THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 6.30 p.m. on Wednesday, December 4th, 1957, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by Dr. J. R. Nicholls, C.B.E., F.R.I.C., Past President.

The meeting took the form of a discussion on "Standardisation" and was opened by R. C. Chirnside, F.R.I.C., L. S. Theobald, M.Sc., A.R.C.S., F.R.I.C., J. Haslam, D.Sc., F.R.I.C., and G. Ingram, A.R.I.C. Owing to Mr. Theobald's absence through illness his contribution was read by Mr. Chirnside.

NEW MEMBERS

Ordinary Members

Ernest Vernon Browett, B.Sc. (Lond.), A.R.I.C.; David Maxwell Brown, B.Sc. (Edin.), F.P.S.; Arthur Clark, B.Sc. (Sheff.); Raymond Thomas Clark; Clive Kear Colwell, M.A. (Cantab.); Eric Leslie Crooks; Ernest George Cummins, B.Sc. (Lond.); Christine Sylvia Delves, B.Sc. (Reading); William Ernest Elstow, B.Sc., Ph.D. (Lond.), A.R.I.C.; Frank Joseph Green, M.Sc. (Lond.), F.R.I.C.; Dennis Francis Harris; George Frederick James Hart; Francis Henry Hillyard, B.Sc. (Birm.); Sidney Louis Kidman, A.R.I.C.; John Weale Macaulay, A.M.C.T.; Nasir Mahmood, B.Sc. (Punjab); Bernard Whitley Elliott Minifie, F.R.I.C.; John Park, B.Sc. (Edin.), Dipl.Tech.Chem., F.R.I.C.; Robert Gray Reid, A.R.I.C.; Brian James Rushton, B.Sc. (Manc.); Rosemary Sales, B.Sc. (Lond.); Ian Robert Scholes, B.Sc. (Lond.), A.R.I.C.; Derek William Skidmore; Jack Thomas; James Raymond Townley; Edmond Albert Underwood; John Oswald Lance Wrigley.

Junior Members

Keith Edward Bicknell, A.I.M.L.T.; Derek Alfred Day; Douglas McDonald Dick, B.Sc. (Aber.); James Alistair Grant; David Ramsden.

SCOTTISH SECTION

An Ordinary Meeting of the Section was held at 7.15 p.m. on Wednesday, October 30th, 1957, in the Central Hotel, Glasgow. The Chair was taken by the Chairman of the Section, Dr. Magnus Pyke, B.Sc., F.R.I.C., F.R.S.E.

A discussion on "The Estimation of Additives to Bread and Flour" was opened by J. Sword, M.A., B.Sc., Ph.D., F.R.I.C. A. N. Harrow, A.H.-W.C., F.R.I.C., and H. C. Moir, B.Sc., F.R.I.C., also contributed. The meeting then continued with a general and informal discussion.

WESTERN SECTION AND MICROCHEMISTRY GROUP

A joint Meeting of the Western Section and Microchemistry Group, together with the South-Western Counties Section of the Royal Institute of Chemistry, was held on Friday and Saturday, September 27th and 28th, 1957, in Exeter.

On Friday afternoon a visit was paid to the Silverton Paper Mills of Messrs. Reed & Smith Ltd. At 5.15 p.m. there was a meeting in the Washington Singer Laboratories of the University of Exeter on "Some Applications of Microchemistry." The Chair was taken by Mr. D. F. Phillips, F.R.I.C., Chairman of the Microchemistry Group, who thanked Mr. E. Bishop, B.Sc., A.R.T.C., A.R.I.C., of the University of Exeter, for his work in the organisation

of the meeting. The following papers were presented and discussed: "Applications to Paints and Pigments," by C. Whalley, B.Sc., F.R.I.C. (see summary below); "Applications to Soils and Fertilisers," by B. M. Dougall, M.Sc., F.G.S., A.R.I.C.

After a social programme on Saturday, September 28th, there was a Discussion Meeting on "The Use and Abuse of Microchemistry," which was introduced by C. L. Wilson, D.Sc., Ph.D., F.R.I.C., and S. Bance, B.Sc., A.R.I.C.

Some Applications of Microchemistry: Application to Paints and Pigments

Mr. C. Whalley selected three topics for detailed discussion, as follows—

(*i*) the semi-micro qualitative analysis of pigment systems;
(*ii*) the analysis of small amounts of gases;
(*iii*) the techniques used to examined the various types of blemishes that occasionally appear on painted surfaces.

In the first example a new scheme for the qualitative analysis of white pigments and extenders on the milligram scale was described. Classical analytical schemes were not easily applicable to this type of system owing to the intractable nature of the materials present, and further information was required about the composition of the system than could be obtained by finding the elements present. The new scheme avoided fusions, relying on the limited solubility of the materials in acids of increasing strength. The materials were divided into groups based upon their solubilities in dilute nitric, concentrated hydrochloric, concentrated sulphuric acid and concentrated phosphoric acids and sodium hydroxide solution. Sensitive colour tests were used to detect the various elements after limited separations. The whole scheme was set in the form of a working board, and mixtures up to and including sixteen components could readily be analysed.

The second example was illustrated by an improved apparatus for the analysis of about 0·3 ml of gas samples. The method used was based upon classical techniques, but solid bead reagents were used as absorbers. All the gas holders, reagent bead holders, sparking wires and transfer pipettes were securely mounted, but could easily be brought into action in the gas chamber and measuring burette. The whole apparatus had been designed to be very simple and foolproof in operation.

Finally, the techniques developed to examine the various stains, inclusions, blooms, crystals and so on that occasionally appear on painted surfaces were described. These could cause deterioration in the appearance of the paint film and might even cause the adhesion to fail. The amount of material available for this detective work was usually very small, and the whole of the work was carried out under the microscope at a magnification of 50 × with the help of a pair of micro dissector units. A variety of special cutting tools, needles, remote operated forceps, and so on, for removing the material for investigation were described, together with a micro fusion device and "wet box" for carrying out chemical tests. These latter were performed in ground depressions on a microscope slide, in hair capillaries, on treated papers and on the surface of single beads of ion-exchange resins suitably treated. Most of the work was qualitative and tests were described for the identification of the common types of blemishes.

WESTERN SECTION and PHYSICAL METHODS GROUP

A joint Meeting of the Western Section and the Physical Methods Group was held at 6.30 p.m. on Friday, October 25th, 1957, in the Kings Head Hotel, Newport, Monmouthshire. Mr. P. J. C. Haywood, B.Sc., F.R.I.C., Chairman of the Western Section, opened the meeting, welcoming the Physical Methods Group to Newport and then invited Dr. J. E. Page, F.R.I.C., Chairman of the Physical Methods Group, to take the Chair.

The following papers on "Flame Photometry" were presented and discussed: "Atomic Absorption Spectroscopy," by A. C. Menzies, M.A., D.Sc. (see summary below); "Recording Flame Photometry," by L. Brealey, B.Sc. (see summary below).

The meeting was preceded at 2.15 p.m. by a visit to the factory of Monsanto Chemicals Ltd.

Atomic Absorption Spectroscopy

Dr. A. C. Menzies said that, since Wollaston's discovery of the absorption lines, known as Fraunhofer lines, in the spectrum of the sun, little use had been made of the

phenomenon for analytical purposes. A. Walsh in Australia had drawn attention to atomic absorption as a means of analysis, had made appropriate apparatus, and had used it more especially for the measurement of oscillator strengths, of great importance in the theory of atomic emission and absorption processes.

At first it had been hoped that the method would be generally applicable, but conditions had not so far been found for that. Elements fell broadly into three classes: Some, like magnesium, zinc and copper, were very sensitive; others, like iron and chromium, were only moderately sensitive; some, like aluminium, were extremely insensitive. The explanation of this was not straightforward, and there might be a number of causes.

A second point of interest was the curvature of the working graphs produced by plotting the optical density of the flame against concentration of the metal being sprayed into the flame. Near the origin, *i.e.*, for low concentrations, the graph was linear, but it changed slope eventually, tending to become more parallel to the axis of concentration.

These matters were being worked upon, and the speaker stated the latest views on them. He also gave some account of uses to which the equipment could be put.

Recording Flame Photometry

Mr. L. Brealey said that flame photometry had been established as one of the most useful of analytical techniques, and, as hotter flames were used, the number of elements that could be determined was increasing.

As spectra became more complex, recording instruments could be used with great advantage, both for developing analytical methods and for routine use. Such instruments were quite simple, and any good flame spectrophotometer could be easily adapted for recording purposes. One of the most useful applications had been a study of the changes in flame background that occurred with variation in sample constitution. Many of the reported cationic interferences could be attributed to this change in background, and by using recorded spectra it was a simple matter to eliminate this variable.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Thursday, October 24th, 1957, in the Gas Showrooms, Nottingham. The Chair was taken by the Vice-Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following paper was presented and discussed: "The Analytical Chemistry of Morphine Poisoning," by A. S. Curry, M.A., Ph.D.

MICROCHEMISTRY GROUP

The eleventh London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, October 30th, 1957, in "The Feathers," Tudor Street, London, E.C.4. Dr. G. F. Hodsman, A.Inst.P., took the Chair.

A discussion on "British Standards in Microchemistry" was opened by C. Meredith and G. Ingram, A.R.I.C.

Quantitative Inorganic Chromatography

Part III.* The Separation and Determination of Ferrous and Ferric Iron

By F. H. POLLARD, J. F. W. McOMIE, A. J. BANISTER and G. NICKLESS

The method of selecting a solvent system suitable for the separation of various metals in different valency states is described. The optimum composition of this solvent system for the separation of ferrous and ferric iron has been investigated and a detailed study made of the experimental variables that affect this separation.

A comparative study has also been made of methods for the quantitative determination of ferrous and ferric iron after separation by paper chromatography. The preferred method involves extraction of the metal from the paper and colorimetric measurement after complexing with 2-nitroso-1-naphthol-4-sulphonic acid.

The ratios of iron^{II} to iron^{III} investigated were between 25 to 1 and 1 to 49, and the method was found to be applicable to amounts ranging between 25 and 1250 μg of iron^{II} and between 25 and 2450 μg of iron^{III}. The maximum error expected for any single determination was between 1 and 4 per cent., depending upon the amounts present and the ratio of iron^{II} to iron^{III}.

A new type of graduated capillary dropper that delivers, almost instantaneously, minute amounts of liquid (about 1 to 3 μl) is described. The application of the new chromatographic technique to the determination of iron in mixtures with chromium, manganese, cobalt, nickel and copper is indicated. Salicylaldehyde is used as a sensitive spray reagent for the detection of manganese.

Part 1. The Selection of a Solvent for the Optimum Separation of Ferrous and Ferric Iron by Paper Chromatography

Although much work has been done in the last few years on the paper-chromatographic separation of mixtures of different metal cations, the separation of the ions of a metal according to their valency states has received much less detailed attention. Separations of various ionic species of individual polyvalent metals have, however, been reported for antimony,[1] arsenic,[1,2,3] chromium,[1,4] cobalt,[1] copper,[1,5,6] iron,[5,7,8,9] mercury,[1,5,7,9,10,11] molybdenum,[1,9,12,13,14] platinum,[1] plutonium,[15] thallium,[9,16,17] uranium[8,17] and vanadium.[1]

Part 1 of this paper describes the selection of a general solvent for the separation, on the same chromatogram, of the valency states of many metals as chlorides,[1] the modification of this solvent (by a semi-graphical method) to give the optimum separation of ferrous and ferric ammonium sulphates, and a study of the experimental variables that affect this separation.

Selection of general solvent

The components of the chromatographic solvent system, ether, methanol, water and hydrochloric acid, were selected for the following reasons.

Aqueous hydrochloric acid was chosen because (*a*) as is well known, the anion and cation of a salt may move independently of each other when eluted on a chromatogram, depending on the nature of the mineral acid present in the solvent. Doubling of the spot is liable to occur if the acid of the solvent is much weaker than the acid from which the solute is derived, *e.g.*, ferric chloride produces two spots in the solvent system *n*-butanol - glacial acetic acid - water (5:4:1) on acid-washed paper.[18] Also (*b*) the sensitivity of Fe^{2+} and U^{4+} to atmospheric oxidation rapidly diminishes with decreasing pH, and (*c*) ferric chloride and uranyl chloride tend to show high R_F values in chromatographic solvents containing high concentrations of hydrochloric acid together with ether, lower alcohols or ketones. For iron^{III}, this is due to the solubility of the complex formed between ferric chloride and the excess of hydrochloric acid.

* For particulars of Part II of this series, see reference list, p. 799.

Methanol and ether were chosen (*a*) because the low viscosity of methanol assisted in rapid separation and (*b*) because, by the use of solvents prepared by mixing a polar solvent with various proportions of a non-polar solvent, the R_F values of ions and the quality of separations could be systematically varied.

The best ratio of methanol to ether was found by adding various amounts of methanol (between 10 and 50 ml) to a mixture of 1 ml each of water and concentrated hydrochloric acid, sp.gr. 1·18, and 50 ml of ether, and then plotting graphs of the R_F values for each metal and valency state against the ratio by volume of methanol to ether. In general, R_F values rose steadily as the methanol - ether ratio increased, and the widest R_F separations occurred at ratios between 1 to 1·6 and 1 to 2. (The solvent system ether - acetone - water - concentrated hydrochloric acid was also investigated, but the iron^{III} spot showed appreciable tailing.) With 30 ml of methanol and 50 ml of ether, the volumes of water and concentrated hydrochloric acid were varied as in the example shown in Fig. 2. The solvent finally selected for the separation of the valency states of iron, chromium, molybdenum, uranium and other metals was composed of ether, methanol, water and concentrated hydrochloric acid in the ratio 50:30:15:4.[1,8]

The selection of a modified solvent by a semi-graphical method

The general solvent was modified after an examination of the results of the experiments with the general solvent discussed above. Fig. 1 shows the saturation curve of 50 ml of ether *plus* 30 ml of methanol with aqueous hydrochloric acid at 18° C; any point within the area bounded by the axes and the curve corresponds to a homogeneous mixture, while points outside this area correspond to mixtures that give two phases.

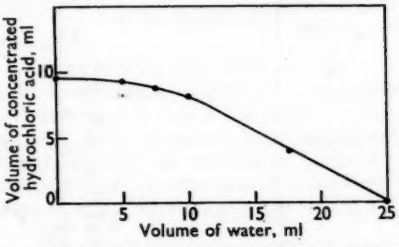

Fig. 1. Saturation curve for 50 ml of ether and 30 ml of methanol

Experimental chromatograms—

Effects of water and acid concentrations—The effects on the chromatogram of variations in water and acid concentrations are shown in Fig. 2. In each experiment the volume of ether was 50 ml and that of methanol was 30 ml, and all mixtures were homogeneous. Iron was applied as 1·40-μl spots of a solution of ammonium ferrous or ferric sulphate in 0·25 or 0·5 *N* sulphuric acid, respectively, and a 1 + 1 mixture of the two solutions (all solutions containing 5 μg of iron per μl); these spots were placed 2 cm apart on the starting line of each sheet of unwashed Whatman No. 1 filter-paper. The times of jar equilibration before elution were 70 minutes $\pm$ 1 minute and the times of run were 2 hours 30 minutes $\pm$ 2 minutes at 16·8° $\pm$ 0·5° C. All other details of the ascending elution technique were as described later for the investigation of factors affecting the separation of ferrous and ferric iron (p. 783).

When the chromatograms were dry, the solvent front, indicated by a bluish white fluorescence in ultra-violet light, was marked on each chromatogram, and the spots were revealed by spraying with 0·5 per cent. aqueous potassium ferricyanide.[8] Since the purpose of the investigation was to find the solvent that gave the most rapid and complete separation of ferrous and ferric iron, the chromatograms were run for a fixed time and the distance moved by the spots was plotted against acid concentration at 4, 8 and 12 ml of water (Fig. 2). This figure shows that the best separation took place with 8 ml of water and 6 ml of acid. A similar conclusion was also reached when the results were plotted in terms of R_F instead of distance.

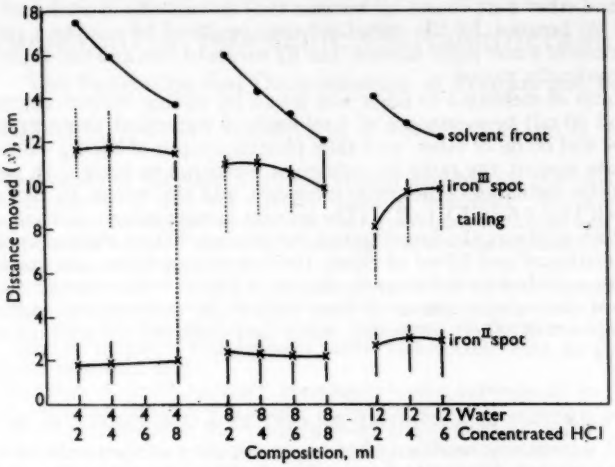

Fig. 2. Variation of distance moved in 2½ hours by iron spots with change in volumes of water and acid; crosses denote the centre of gravity of each spot and dotted lines indicate forward or backward tailing of the spots

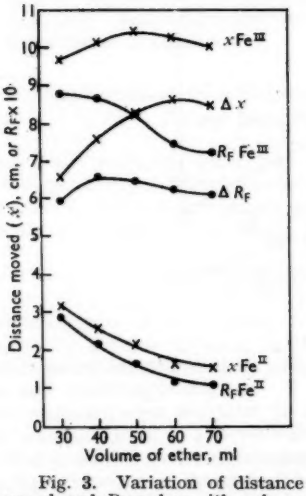

Fig. 3. Variation of distance moved and R_F value with volume of ether; volume of methanol, 30 ml

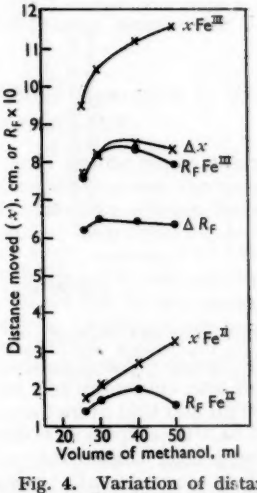

Fig. 4. Variation of distance moved and R_F value with volume of methanol; volume of ether, 50 ml

In Figs. 3 and 4: volume of water, 8 ml; volume of concentrated hydrochloric acid, 6 ml;

x Fe^{II} = distance moved by the centre of gravity of the iron^{II} spot;

R_F Fe^{II} = (R_F value of iron^{II}) × 10;

$\Delta x = x$ $Fe^{III} - x$ Fe^{II};

x Fe^{III} = distance moved by the centre of gravity of the iron^{III} spot;

R_F Fe^{III} = (R_F value of iron^{III}) × 10;

$\Delta R_F = R_F$ $Fe^{III} - R_F$ Fe^{II}

Effects of ether and methanol concentrations—With 8 ml of water and 6 ml of concentrated hydrochloric acid, the volumes of ether and methanol were varied as shown in Figs. 3 and 4. Methanol contents below about 26 ml could not be investigated, since 26 ml of methanol lies

just above the minimum quantity that produces a homogeneous mixture with 50 ml of ether *plus* 8 ml of water *plus* 6 ml of concentrated hydrochloric acid. Except for the time of equilibration, which was 1 hour 40 minutes ± 5 minutes, and the temperature, which was 17·4° ± 0·3° C, the experimental conditions were those used in investigating variations in water and acid content.

RESULTS—

Choice of volume of ether—The curves of composition against distance moved (x) and R_F (Fig. 3) indicate that the widest separation occurred at 60 and 40 ml of ether, respectively. If the permitted length of run were unlimited, the peak in the Δx curve would have been chosen as giving the best solvent, but since the small size of the apparatus restricted the length of run, 50 ml of ether was chosen as a suitable compromise between these two results. If a solvent with maximum R_F separation is required, graphs may be plotted of composition against R_F for chromatograms with constant length of run.

Choice of volume of methanol—The curves of composition against R_F and x (Fig. 4) indicate that the best separation occurred close to 35 ml of methanol, but the maximum separation (in terms of x) of the adjacent extremities of the spots occurred at 30 ml of methanol. Hence the modified solvent as finally chosen was composed of ether - methanol - water - concentrated hydrochloric acid in the ratio 50 : 30 : 8 : 6 v/v.

This method of designing solvents for quantitative separations in which rapidity is stressed together with maximum R_F separation has been applied to other metal valency states.[19] The semi-graphical method has also been found useful in selecting solvents for separating complex mixtures of closely related compounds, *e.g.*, the series of sodium silver thiosulphate complexes.[20]

INVESTIGATION OF FACTORS AFFECTING THE SEPARATION OF THE FERROUS AND FERRIC IRON

EXPERIMENTAL PROCEDURE—

Solvent components were mixed in the order: water, acid, ether, alcohol, in glass-stoppered bottles to prevent evaporation, cooled after the additions of the acid and the alcohol, and finally allowed to stand for about 1 hour to attain room temperature. All solvents were freshly prepared. After about 36 hours, the composition of the solvent had altered, probably by esterification, and this resulted in poorer separations.

The following standard solutions of ferrous and ferric iron were prepared and used—

Ferrous chloride solution (4·95 *μg of iron per μl*)—$FeCl_2.4H_2O$ dissolved in 5 per cent. v/v aqueous hydrochloric acid.

Ferric chloride solution (4·80 *μg of iron per μl*)—$FeCl_3$ dissolved in 5 per cent. v/v aqueous hydrochloric acid.

Mixed chloride solution—A 1 + 1 mixture of the ferrous chloride and ferric chloride solutions.

Ammonium ferrous sulphate solution (5·0 *μg of iron per μl*)—$(NH_4)_2SO_4.FeSO_4.6H_2O$ dissolved in 0·25 *N* sulphuric acid.

Ammonium ferric sulphate solution (5·0 *μg of iron per μl*)—$(NH_4)_2SO_4.Fe_2(SO_4)_3.24H_2O$ dissolved in 0·5 *N* sulphuric acid.

Mixed sulphate solution—A 1 + 1 mixture of the ammonium ferrous sulphate and ammonium ferric sulphate solutions.

The chloride solutions were used with the general (unmodified) solvent and were standardised colorimetrically with 2-nitroso-1-naphthol-4-sulphonic acid according to the method described in Part 2, p. 795. The sulphate solutions were used with the modified solvent. Strips of Whatman No. 1 filter-paper (30·5 × 6·5 cm), cut perpendicular to the machine direction, were marked with a starting line 2 cm from one end, and 1·40-μl spots of the appropriate solutions were applied 2 cm apart on this line from a calibrated capillary dropper (see p. 789).

The apparatus consisted of a gas-jar 30 cm high with an internal diameter of 7·5 cm, two pieces of glass, 14 × 5 cm, ground flat on the 14-cm edges, a cover plate 14 × 9 cm and two elastic bands. The top of the gas-jar was greased and half-covered with one of the pieces of glass, 100 ml of solvent mixture were poured into the jar and the second piece of glass was pressed on to the rim to complete the seal. The cover plate was placed on top and held firmly on the glass slides by the two elastic bands. The liquid was swirled round the sides

of the jar to saturate the atmosphere with solvent vapour, care being taken to prevent the solvent from reaching the grease at the top of the jar. After 1 hour's equilibration of the gas-jar, the cover plate was removed and the glass slides were pulled about 1 mm apart. The filter-paper strip was inserted through the gap until the lower edge of the paper just touched the solvent surface. The cover plate was placed over the protruding upper end of the strip and the elastic bands were passed over the ends of the glass slides and cover plate.[21] The paper strip was left in position for the required length of time or until the solvent front had reached the position marked on the edges of the strip. It was then removed and dried.

Both the solvent front and the acid front (which could also be detected with indicator) were visible in ultra-violet light on the unsprayed chromatograms as a fluorescent band and a thin line, respectively. The iron spots were detected by spraying the chromatogram with 0·5 per cent. aqueous potassium ferricyanide.[8] R_F values were measured for the positions of highest concentration, gauged visually, the probable error being $\pm$ 0·01 on a 15-cm run.

By using this procedure, except where specifically stated otherwise, the following factors affecting the efficiency of the separation were studied: (*a*) volume of solvent in the gas-jar; (*b*) time of equilibration of the gas-jar atmosphere; (*c*) type and treatment of the paper; (*d*) equilibration of the paper; (*e*) position of the spots with respect to the edge of the paper; (*f*) other substances present with the solutes (and hence the drying time); (*g*) distance from solvent level to starting line; (*h*) length of run; (*i*) temperature.

(*a*) VOLUME OF SOLVENT IN THE GAS-JAR—

Conditions—Modified solvent and sulphate solutions; time of gas-jar equilibration, 65 minutes $\pm$ 2 minutes; spots dried for 5 to 10 minutes; time of elution, 2 hours 40 minutes $\pm$ 2 minutes; temperature, $T = 17{\cdot}7° \pm 0{\cdot}5°$ C.

Experiment—Chromatograms were run with 25, 50, 75, 100, 125 and 150 ml of solvent in the gas-jars. The results are plotted in Fig. 5.

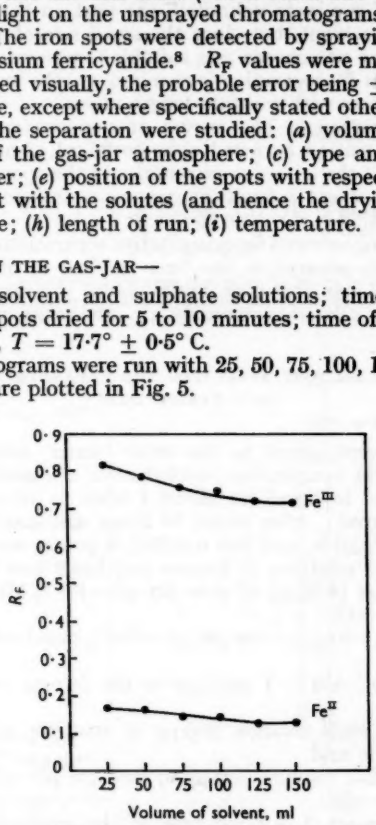

Fig. 5. Variation of R_F value with volume of solvent in the gas-jar

The decrease in R_F value observed with increase in volume of solvent was probably due to (*i*) improved equilibration and (*ii*) the relatively smaller changes in composition of the solvent caused by loss into the paper during elution and in saturating the atmosphere of the gas-jar.

(*b*) TIME OF EQUILIBRATION OF THE GAS-JAR ATMOSPHERE—

Conditions—As for (*a*), but $T = 19{\cdot}1° \pm 0{\cdot}5°$ C and 100 ml of the modified solvent were used.

Experiment—The equilibration time was varied between 3 and 60 minutes. On increasing the equilibration time from 10 to 60 minutes, the R_F values decreased slightly, the total decrease over this range (0·02 for iron^{II} and 0·03 for iron^{III}) being almost within the experimental error. With equilibration times of less than 10 minutes, R_F values were variable—

depending upon how well the jar was shaken. An equilibration time of 30 to 60 minutes is recommended.

The sensitivity of R_F values to poor equilibration appears to be due to evaporation from the chromatogram during elution. This applies particularly to the region between the acid and solvent fronts, which contains a high proportion of ether (see p. 787). When the top of the gas-jar was adequately greased, iron^{II} and iron^{III} had R_F values of 0·14 and 0·72, respectively, but these rose to 0·20 and 0·87 when the top was ungreased. Therefore, although the separation was little affected, the R_F values substantially increased with leakage of solvent vapour from the gas-jar.

One of the important features of the ascending-elution apparatus used is that the chromatogram can be admitted to the gas-jar with very little disturbance of the atmosphere of the jar. This is not true for the apparatus normally used for descending elution, and to obtain reproducible R_F values it is necessary to equilibrate the tank further, and hence the paper, before admitting the solvent to begin elution. Such a procedure is highly undesirable for the separation of iron^{II} and iron^{III} owing to their instability (particularly when dry) when in contact with the paper.

(*c*) TYPE AND TREATMENT OF THE PAPER—

Conditions—General solvent and chloride solutions; spots dried for 10 minutes; $T = 17{\cdot}5° \pm 0{\cdot}5°$ C.

Experiment—Whatman filter-papers Nos. 1, 3MM, 31, 54, 540 and 541 and acid-washed No. 1 (AW.1)[21] were investigated with regard to R_F reproducibility, speed of running and amount of iron impurity. For constant lengths of run, no significant differences in iron^{II} and iron^{III} R_F values were observed. The speed of running was in the order—

$$31 > 54 > 3MM > AW.1 > 541 > 1 > 540.$$

No. 31 paper required 70 minutes for a 15-cm run past the starting line and No. 540 paper, 4 hours 45 minutes. Iron impurities in the paper were detected as a black band at the iron^{III} position by spraying with oxine[22] after elution. Visual comparison of the iron bands showed the order of increasing iron content to be—

$$AW.1 < 540 < 54 < 541 < 1 < 3MM < 31.$$

A comparison was made of R_F reproducibility of unwashed and acid-washed No. 1 paper. Variations of R_F value, especially for iron^{III}, from one strip to another (cut from the same sheet) were found to be appreciably smaller with sheets of untreated paper than with the acid-washed sheets (particularly when they were freshly washed and dried). A 1-month old batch of washed paper approximated the most closely to untreated paper, and so was used in experiment (*d*).

(*d*) EQUILIBRATION OF THE PAPER—

Conditions—General solvent and chloride solutions; time of elution, 3 hours 30 minutes ± 2 minutes; $T = 16{\cdot}7° \pm 0{\cdot}5°$ C.

Experiment—Series of chromatograms were run with 1 hour's equilibration and with no equilibration of the paper both on unwashed and acid-washed Whatman No. 1 filter-paper. Paper equilibration was effected by hanging the spotted and dried strips in covered gas-jars so that the bottom of the strip did not quite touch the solvent surface. After equilibration, the strips were lowered to start the elution.

TABLE I

EFFECT OF EQUILIBRATION OF THE PAPER ON R_F VALUES FOR FERROUS AND FERRIC IRON IN MIXED CHLORIDE SOLUTION

The errors quoted show the maximum deviations from the mean

Paper	Time of equilibration	Mean R_F value for— acid front	ferrous iron	ferric iron	ΔR_F
Unwashed	0	0·875 ± 0·005	0·270 ± 0·020	0·770 ± 0·010	0·500
Unwashed	1 hour	0·830 ± 0·025	0·250 ± 0·020	0·720 ± 0·025	0·470
Acid-washed ..	0	0·870 ± 0·020	0·270 ± 0·020	0·760 ± 0·020	0·490
Acid-washed ..	1 hour	0·815 ± 0·010	0·260 ± 0·015	0·725 ± 0·015	0·465

The results, which are shown in Table I, are the average values from series of four chromatograms run under identical conditions. Although pure ferric chloride was placed on the centre of the starting line, all chromatograms that had had 1 hour's equilibration of the paper also showed faint spots for ferrous chloride in this position. This is believed to have been due to the reduction of the ferric to ferrous chloride by the paper,[2] although it has been suggested that the action of light has this effect.[24] In the experiments with initially unequilibrated paper, equilibration must occur during elution and the results in Table I suggest a decrease in R_F value with increasing rate of elution—other factors, however, also affect the R_F value for different lengths of run.

The results showed that (*i*) there was no major difference between the treated and untreated paper, (*ii*) the best separations occurred on unequilibrated unwashed paper and (*iii*) a decrease in R_F values and separation of iron^{II} and iron^{III} when the paper was equilibrated; the reproducibility of the results on unwashed paper was not improved. It was for these reasons that unequilibrated paper was used in the search for the solvent that was to be used for quantitative work on acid-washed paper.

(*e*) POSITION OF THE SPOTS WITH RESPECT TO THE EDGE OF THE PAPER—

On runs very close to the edge of the paper, larger R_F values were obtained, particularly for iron^{III} (see Fig. 6). Trumbore and Rogers[25] and Almássy and Dazsö[26] have also stated that R_F values may vary with the width of the chromatographic strip.

(*f*) OTHER SUBSTANCES PRESENT WITH THE SAMPLE TO BE CHROMATOGRAPHED—

Incomplete drying of the spot on the starting line resulted in increased iron^{II} R_F values and decreased iron^{III} R_F values. This was almost certainly due to the modification of the solvent's properties as it passed over the damp spot on the starting line, as an increase in water concentration in the solvent was found experimentally to have a similar effect.

(*g*) DISTANCE FROM SOLVENT LEVEL TO STARTING LINE—

Various investigators,[27,28,29,30,31] particularly in organic chromatography, have demonstrated that R_F values vary with the distance between the solvent source and the spot of solute on the starting line. This has usually been attributed to effects of solvent-composition gradients on the chromatogram, particularly of water, and the work now described supports this conclusion.

Conditions—Modified solvent and sulphate solutions; time of gas-jar equilibration, 60 minutes $\pm$ 2 minutes; spots dried for 25 to 30 minutes; d varied between 1 and 10 cm; l was 12·0 $\pm$ 0·2 cm; $T = 17{\cdot}7° \pm 0{\cdot}5°$ C.

Experiment—In these chromatograms, d is the distance from the starting line to the lower end of the strip and l is the length of run from starting line to solvent front. The chromatograms were placed in the gas-jars so that the tip of the paper just touched the solvent surface. During elution, the chromatogram swelled so that, at the end of a 15-cm run, the tips of the strips were about 5 mm below the solvent level. The distance, d, is therefore marked off before elution. Results are plotted in Fig. 7.

Very faint backward tailing of iron^{III} increased in extent and intensity with increasing values of d, so that at $d = 8$ cm it reached the iron^{II} R_F value, where a definite spot could be discerned. This tailing was probably due to reduction of iron^{III} to iron^{II} by the paper.

As d increased beyond 4 cm, the points for iron^{II} and iron^{III} approached linearity. At $d = 1$ cm, the R_F value of iron^{III} was not easily reproducible to within the assessed experimental error of $\pm$ 0·01 to 0·02. For minimum tailing, but maximum separation and reproducibility, the best distance from starting line to solvent level was 3·0 cm. However, in quantitative work, where stability of the solutes was more important than high reproducibility, $d = 2$ cm had to be adopted. Descending elution, when d is normally about 3·5 to 5·5 cm, was therefore unsuitable for this work.

It is significant that throughout these runs, and also those at different lengths of run, the iron^{III} spot remained at the acid front. This suggested that little movement of ferric iron took place while the acid-free region (zone 1) ahead of the acid front passed over the iron spot (so that after any movement in zone 1, the spot was very soon overtaken by the acid front), *i.e.*, in a solvent with the composition of zone 1, the R_F value of iron^{III} is very small. In fact, the R_F values of iron^{II} and iron^{III} were shown to be zero in a number of ways—

(*i*) A chromatogram was spotted with ammonium ferrous and ferric sulphates, d was 15 cm and l was marked at 4 cm. The acid front lay behind the starting line throughout the run and only zone 1 passed over the spots. No movement took place.

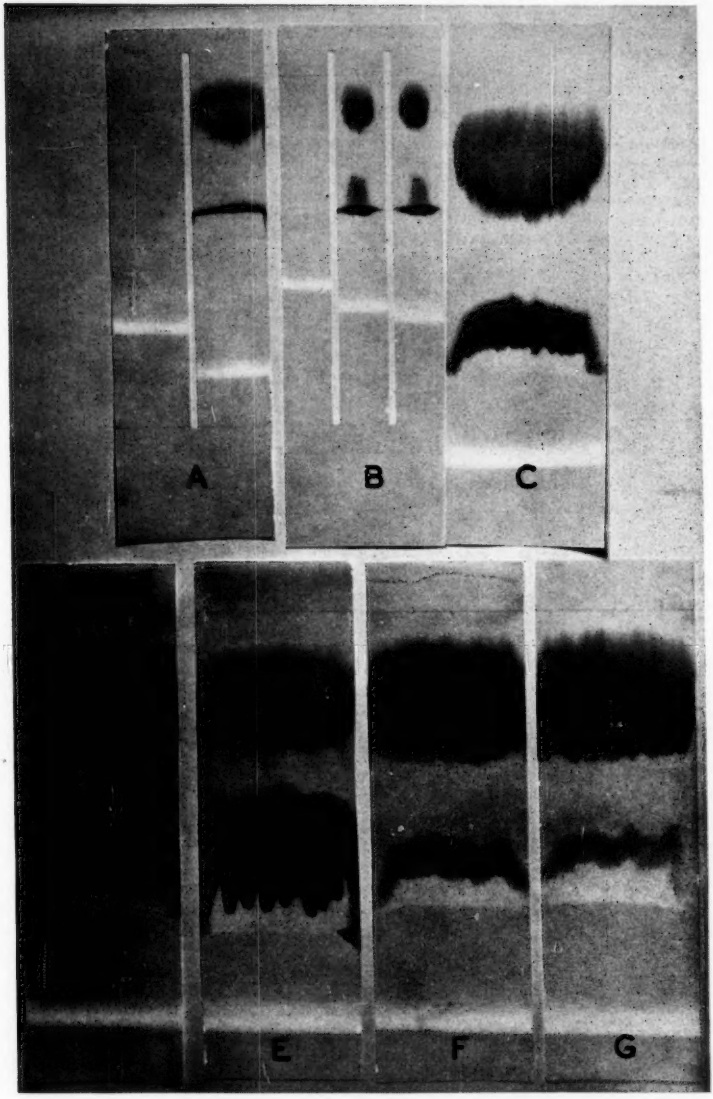

Fig. 6. Quantitative chromatograms photographed in ultra-violet light; starting line marked 2 cm from the end of each chromatogram; a fluorescent band reveals the position of each solvent front:

A,	25	μg of iron^{II}	+	25	μg of iron^{III} (double strip)
B,	27·8	μg of iron^{II}	+	27·8	μg of iron^{III} (triple strip)
C,	250	μg of iron^{II}	+	250	μg of iron^{III}
D,	100	μg of iron^{II}	+	500	μg of iron^{III}
E,	50	μg of iron^{II}	+	1200	μg of iron^{III}
F,	500	μg of iron^{II}	+	100	μg of iron^{III}
G,	1250	μg of iron^{II}	+	50	μg of iron^{III}

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(*ii*) On another strip ($d = 6$ cm; $l = 3$ cm), elution by zone 1 produced no displacement of ferric chloride or ferric sulphate, but a spot of ferric chloride dissolved in a solution containing 5 per cent. v/v of hydrochloric acid moved to R_F 0·4. This indicated that neither ferric chloride nor ferric sulphate moved until they were converted to the hydrochloride complex of ferric chloride by the hydrochloric acid in zone 2.

(*iii*) As a final check, 20 g of washed and dried cellulose powder were packed in a glass column of 2·9 cm internal diameter; the height of the powder in the column was 17·7 cm. Phenol red was dissolved in a portion of the modified solvent mixture to reveal the position of the acid front during elution. The solvent was poured through the dry column and fractions were collected at intervals. The indicator was pink in the acid zone and yellow in the neutral zone. The composition of these small fractions (each 0·5 to 1·5 ml) was determined by gas chromatography.[32] The water concentration in zone 1 was found to depend to some extent on the pre-treatment of the cellulose powder. A solvent composed of ether - methanol - water (59 : 33 : 8), as given by the analysis of the eluate immediately before the arrival of zone 2, represented the solvent from zone 1 most likely to result in the movement of iron on a chromatogram (through having the highest methanol and water concentrations), but when a solvent of this composition was investigated, it produced no movement of ammonium ferrous or ferric sulphates (but only in the absence of hydrochloric acid from the original spots).

(*h*) LENGTH OF RUN—

In conditions similar to those described in (*g*) above, R_F values were obtained for lengths of run 4 to 20 cm and $d = 2$ cm (Fig. 8). As discussed in (*g*), these variations in R_F are principally the effect of solvent-composition gradients on the chromatogram.

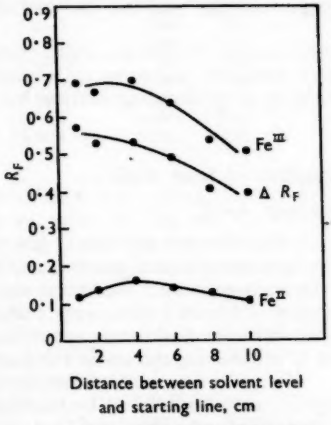

Fig. 7. Variation of R_F value with distance, d, between the solvent level and the starting line; $l = 12 \pm 0·2$ cm

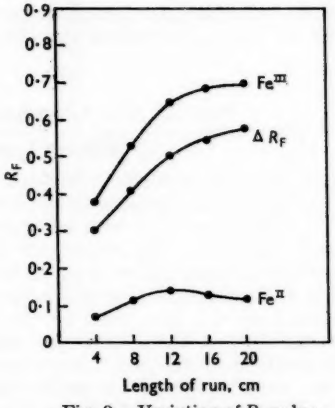

Fig. 8. Variation of R_F value with length of run, l; $d = 2$ cm

In Figs. 7 and 8: ΔR_F = (R_F value of iron^{III}) − (R_F value of iron^{II})

(*i*) TEMPERATURE—

Fluctuations in R_F over the temperature range 15° to 20° C lay within the experimental error.

THE POSITIONS OF THE AMMONIUM AND SULPHATE IONS

By spraying the chromatogram with lead cobalt nitrate - sodium nitrite reagent,[33] the ammonium ion was found at R_F 0·12, and by spraying with 0·2 *N* aqueous barium chloride followed by aqueous rhodizonic acid (sodium salt), a white spot appearing on a pink background showed the sulphate ion to be at R_F 0·72. On these chromatograms iron^{II} was at R_F 0·17 and iron^{III} at R_F 0·82.

With small spots of iron^{III} (*e.g.*, 10 μg) very faint backward tailing reached its maximum concentration at the position of the sulphate ion. This suggested that the iron^{III} was giving double spots owing to the competition between the two anions (SO_4^{2-} and Cl^-) for the cation (Fe^{3+}), but this conclusion was shown to be incorrect when the replacement of ammonium ferric sulphate by ferric chloride to avoid such competition did not eliminate the phenomenon.

Since this effect was not serious and was not evident in the quantitative work, the cause was not further investigated.

Conclusions

Under the optimum conditions—100 ml of freshly prepared solvent, ether - methanol - water - concentrated hydrochloric acid (50:30:8:6 v/v) at 17° ± 2° C, equilibrated in the apparatus for 60 minutes ± 5 minutes, and with spots of ammonium ferrous and ferric sulphates in 0·25 and 0·5 *N* sulphuric acid, respectively (1·40 μl of solution containing 5 μg iron per μl), dried in air for 5 to 10 minutes on strips cut from a single sheet of Whatman No. 1 filter-paper, no paper equilibration, $d = 2$ cm and $l = 15$ cm—the R_F values of ferrous and ferric iron were found to be 0·17 and 0·82, respectively. The spots lay within the R_F limits 0·08 to 0·22 (iron^{II}) and 0·77 to 0·85 (iron^{III}). On any one sheet of paper, under these conditions, R_F values were easily reproducible to within ± 0·02, but quite large differences (*e.g.*, within a range ± 0·05) were found between chromatograms run under similar conditions, but on different occasions and with different batches of paper. This is evident on comparing the points for similar conditions in the figures showing the results of investigations. When these investigations were repeated, the shapes of these graphs and the conclusions drawn from them remained fully reproducible. Hence, in an investigation into the effect of any experimental variable on a chromatographic separation, it is strongly advisable to cut the strips from the same sheet of paper and to run the chromatograms concurrently.

The detailed examination of the solvent under wide ranges of experimental conditions showed its suitability for quantitative determinations of unknown mixtures of ferrous and ferric iron. This examination also showed the unsuitability of descending elution for such work.

Part 2. The Colorimetric Determination of Iron with 2-Nitroso-1-naphthol-4-sulphonic Acid

The analytical procedure adopted was as follows. The ferrous and ferric iron were applied to the paper strip as a band of mixed ammonium ferrous and ammonium ferric sulphates. The separation was effected by ascending chromatography. The time required for elution rarely exceeded 2½ hours and often shorter times of about 1 hour were sufficient.

In previous quantitative work on other metals[34] some difficulty had been encountered in designing a chromatogram in which the pilot strip gave a reliable indication of the position of the metal on the strip used for the determination. To avoid any such difficulties, the positions of the iron bands on a quantitative chromatogram were established by holding the chromatogram in a gas-jar of ammonia vapour immediately after elution. Green ferrous hydroxide rapidly turned brown by atmospheric oxidation, and the dark bands of each valency state showed up clearly in ultra-violet light. The iron in the excised bands was extracted with hydrochloric acid and determined colorimetrically with 2-nitroso-1-naphthol-4-sulphonic acid, sodium salt. Ten to fifteen samples of ferrous *plus* ferric iron could be completely determined in 5 to 6 hours.

Experimental

Pre-treatment of the paper—

Ten sheets of Whatman No. 1 filter-paper, 56 × 23 cm, were steeped in 2½ litres of diluted AnalaR hydrochloric acid (1 + 4, v/v) for 1 week, and the process was then repeated once. The inside sheets from the first washing were arranged on the outside for the second. Regular agitation each day, *e.g.*, for approximately 5 minutes every few hours, improved the extraction.

Preparation of the solutions for the chromatograms—

Stock solution of ammonium ferrous sulphate (25 *mg of iron per ml*)—43·890 g of AnalaR ammonium ferrous sulphate, $(NH_4)_2SO_4.FeSO_4.6H_2O$, were dissolved in 250 ml of dilute sulphuric acid (containing 25 ml of 50 per cent. w/w sulphuric acid).

Stock solution of ammonium ferric sulphate (12·5 *mg of iron per ml*)—Some difficulty was experienced in dissolving sufficient AnalaR ammonium ferric sulphate,

$(NH_4)_2SO_4.Fe_2(SO_4)_3.24H_2O$, to produce a concentration of 25 mg of iron per ml. Therefore, 26·986 g were dissolved in 250 ml of dilute sulphuric acid (containing 25 ml of 50 per cent. w/w sulphuric acid) to give a solution of half the desired concentration. Gravimetric standardisation of this solution by precipitation as ferric hydroxide and ignition to Fe_2O_3 gave 12·50 mg of iron per ml.

Freshly redistilled water was used in the preparation of both ferrous and ferric stock solutions.

Mixed solutions—Standard solutions containing both ferrous and ferric iron were prepared by putting suitable volumes of the stock solutions into 60-ml stoppered bottles and diluting to 50 ml with freshly redistilled water. The ratios of ferrous to ferric iron investigated and the volumes of the stock solutions required for each standard mixed solution are shown in Table II. The amount of iron present in 0·1 ml of mixed solution is tabulated for

TABLE II

RATIO OF FERROUS TO FERRIC IRON IN STANDARD MIXED SOLUTIONS

Ratio of Fe^{II} to Fe^{III}	Fe^{II} in 0·1 ml, μg	Fe^{II} in 50 ml, mg	Volume of 25 mg per ml solution, ml	Fe^{III} in 0·1 ml, μg	Fe^{III} in 50 ml, mg	Volume of 12·5 mg per ml solution, ml	Volume of mixed solution put on chromatograms, ml
1 to 1	100	50	2	100	50	4	0·025, 0·5, 0·1
	500	250	10	500	250	20	0·1
1 to 5	100	50	2	500	250	20	0·1
1 to 10	100	50	2	1000	500	40	0·1
1 to 24	50	25	1	1200	600	48	0·1
1 to 49	50*	12·5	0·5	2450*	612·5	49	0·2
5 to 1	500	250	10	100	50	4	0·1
10 to 1	1000	500	20	100	50	4	0·1
25 to 1	1250	625	25	50	25	2	0·1

* In 0·2 ml.

each valency state, since this was the volume applied to all chromatograms (except at ratio 1 to 49, when 0·2 ml was used). It would have been possible to have prepared stronger solutions at some of the ratios close to 1 to 1 so that a smaller volume could have been taken for the chromatogram, but this would have unnecessarily introduced another variable. The ferrous and ferric iron were applied to the chromatograms from mixed solutions and not in separate applications in order to simulate the conditions of an actual investigation.

The 1 + 1 solution used for the investigation with the capillary dropper contained 5 ml of stock Fe^{II} solution *plus* 10 ml of stock Fe^{III} solution diluted to 20 ml, *i.e.*, 6·25 μg of each valency state per μl.

Binary 1 + 10 mixtures of iron with chromium, manganese, cobalt, nickel or copper—Mixtures of iron with a ten-fold excess of a second metal were prepared from the stock standard ammonium ferric sulphate solution and weighed quantities of AnalaR chromic potassium sulphate, $Cr_2(SO_4)_3.K_2SO_4.24H_2O$, manganese sulphate, $MnSO_4.4H_2O$, cobalt sulphate, $CoSO_4.7H_2O$, nickel sulphate, $NiSO_4.7H_2O$ and copper sulphate, $CuSO_4.5H_2O$. The iron concentration in each solution was 100 μg of iron per 0·1 ml.

DESIGN AND CALIBRATION OF CAPILLARY DROPPERS—

The design of the capillary dropper has been described elsewhere.[22] It consisted of a capillary tube of 0·6 mm internal diameter and 7 mm external diameter, joined to a stem of glass tubing 10 cm long, of 5·5 mm internal diameter and 7 mm external diameter. The height to which the solution rose in the capillary was measured and the capillary tube was cut so that a length shorter than the measured rise remained attached to the stem; this length depended upon the capacity required. The tip of the capillary tube was ground to as fine a point as possible.

The capillary dropper was calibrated by an aqueous and a mercury method. The slight difference between the results by the two methods was attributed to the difference in the shapes of water and mercury menisci and so the result by the first method was used for aqueous solutions.

Aqueous calibration—The surface of the standard ammonium ferrous sulphate solution (containing 25 mg of iron per ml) was just touched with the tip of the freshly cleaned and dried dropper. The liquid inside the capillary immediately rose to the top and the liquid

outside the capillary rose a little above the tip, which was then withdrawn slowly from the solution; this minimised the quantity of liquid adhering to the outside of the dropper. Because the tip was ground, the solution on its outside rapidly evaporated and consequently did not interfere with the calibration. The filled dropper was then placed in contact with a piece of acid-washed Whatman No. 1 filter-paper, of area 1 sq. cm, for about 5 seconds to ensure complete drainage. Provided there was no entrapped bubble of air in the capillary column, the liquid flowed completely into the paper immediately on contact. This procedure was repeated on three more pieces of paper. The amount of iron on each piece of paper was determined colorimetrically by the procedure described on p. 795, and the results were 56·2, 55·9, 54·9 and 55·5 μg of iron (mean, 55·6 μg). Since the original solution contained 25 μg of iron per μl, the volume of the dropper was calculated to be 2·22 μl $\pm$ 0·76 per cent.

PREPARATION OF THE SOLVENT—

The components of the chromatographic solvent—water, concentrated hydrochloric acid, ether and methanol (8:6:50:30)—were added in that order to a glass-stoppered bottle, which was cooled after the addition of the acid and after the final addition of alcohol. Immediately after mixing the temperature usually rose to 28° to 30° C, making it necessary to prevent evaporation by cooling to room temperature in a closely stoppered bottle. Between 75 and 100 ml of solvent were poured into each gas-jar, which was left for 30 to 60 minutes to come to equilibrium before the chromatogram was put in. No temperature control was necessary between 15° and 20° C, though above 20° C forward tailing of ferric iron at the edges of the chromatogram started to be troublesome.

CHROMATOGRAMS—

Chromatograms of three designs were used in the quantitative work (see Fig. 6).

(*i*) A paper strip, 30·5 $\times$ 6·6 cm, was marked with a starting line 2 cm from one end. Starting 1 cm from this end, two slots, 15 $\times$ 0·3 cm, were cut out of the paper so that for 15 cm it was equally divided into three strips, 15 $\times$ 2 cm. This type of triple-strip chromatogram was used for work in which the spots were placed on the starting line from a capillary dropper. Two of the strips were used for a separation and the third strip was used as a blank.

(*ii*) A paper strip was marked and cut in the same way except that only one control slot was cut, leaving two strips, 15 $\times$ 3·15 cm. This type of double-strip chromatogram was used for separations of solutes contained in less than 0·05 ml. One strip was used for the separation and the other for the blank.

(*iii*) A paper strip, 30·5 $\times$ 6·5 cm, with the starting line marked 2 cm from one end. This type of chromatogram was used for all separations involving volumes of solution from 0·05 to 0·2 ml. The blank was run on a separate strip.

METHODS OF DETERMINING AND EXTRACTING SUBSTANCES FROM FILTER-PAPER CHROMATOGRAMS

There are two main procedures used for the determination of substances after elution in paper chromatography: (*i*) extraction techniques, followed by standard microanalytical procedures and (*ii*) *in situ* determinations. Extraction techniques are generally accounted to be the more accurate, although some *in situ* light-absorption methods have an accuracy approaching extraction procedures.[35] Lacourt, Sommereyns and Wantier[36] quote $\pm$ 4 per cent. as the expected accuracy of the spectrophotometric determination, with disodium catechol-3:5-disulphonate, of 10 μg of iron that has been chromatographically separated from other metals on untreated Whatman No. 1 filter-paper and extracted for 90 minutes with 0·25 per cent. w/v sulphuric acid. On account of the accuracy required in the work described in this paper, an extraction method was chosen out of the many that were investigated.

EXPERIMENTAL—

For the preliminary investigation into the efficiency of extraction, the iron was determined by measuring the red colour produced by ferric iron in alkaline medium of pH 7 to 10 with disodium catechol-3:5-disulphonate.[37] The disadvantages of this method were (*a*) its low sensitivity and (*b*) the need for oxidation of iron in the ferrous state to ferric. For these reasons, attention was paid to the reagent 2-nitroso-1-naphthol-4-sulphonic acid, described in the next section on the colorimetric determination of iron.

Various aliquots of a solution containing 25 to 250 μg of ferric iron, delivered from a micrometer syringe, were absorbed into sections of acid-washed Whatman No. 1 filter-paper,

of area 25 sq. cm, and allowed just to dry. The paper was then treated by the extraction method under investigation.

Cold leaching in 15 ml of 1 per cent. v/v acid (but using AnalaR hydrochloric acid instead of formic acid) and washing with an equal volume of water after the method of Martin[38] gave very low results—approximately 50 per cent. recovery on 100-μg samples.

Micro Soxhlet extraction[39] was investigated, glass crucibles fitted at one end with Jena No. 1 glass frits being used in an all-glass apparatus, since previous work had established the presence and difficult removal of many heavy metals in trace quantities in micro-extraction thimbles. Extraction with 1 per cent. v/v hydrochloric acid or 2·5 per cent. v/v sulphuric acid for up to 3 hours gave low results.

Complete wet digestion of the paper by conventional means for destroying organic material[40] usually gave quantitative recovery. A disadvantage of the wet-ashing technique is the long time required to complete the digestion (about 3 to 4 hours) in order to obtain white ash residue. This method was therefore rejected.

Ashing the paper in a crucible was not tried, since it has been reported that this leads to low results. Kolier and Ribaudo,[41] when determining iron by paper chromatography, used an elevated-temperature leaching procedure, which we investigated, modified and adopted. This modified procedure was used in conjunction with both colorimetric methods of analysis (that with disodium catechol-3:5-disulphonate and that with 2-nitroso-1-naphthol-4-sulphonic acid) and the recovery was 99 to 101 per cent., even on 25-μg samples. With each batch of determinations, an equal area of similarly purified paper was used in a blank determination. The order of magnitude of the iron left in the paper after this leaching process was 0·1 μg per sq. cm.

The colorimetric determination of iron

The grouping –CO–C(=NOH)– as contained in the monoximes of diketo compounds is of considerable analytical importance, since it readily forms inner complex salts with many heavy-metal salts.[42] These complexes are generally only partially soluble in water, and extraction into an immiscible organic solvent is necessary before colorimetric determination. When the grouping is incorporated into an aromatic ring system, it still retains the ability to form inner complex salts, and when sulphonic acid groups are substituted into the aromatic ring of such a complexing agent, the product so formed (and its metal complexes) is generally more water-soluble. Nitroso-R salt (disodium 1-nitroso-2-hydroxynaphthalene-3:6-disulphonate) is an important reagent for the spectrophotometric determination of cobalt,[43,44] and has also been used for the colorimetric determination of ferrous iron.[45,46] The reaction between ferrous iron and nitroso-R salt was critically studied by Griffing and Mellon,[47] who found that the sensitivity was much greater than with 1:10-phenanthroline, but that the method suffered from three main disadvantages: (*i*) the intense yellow-green colour of the reagent; (*ii*) its sensitivity to changes of pH and (*iii*) the time required for full development of the green colour of the complex.

Spectrophotometric study of the reaction of ferrous and ferric iron with 2-nitroso-1-naphthol-4-sulphonic acid

Many coloured complexes are formed in reactions between iron, cobalt, nickel and copper salts with the various nitrosonaphtholmonosulphonic acids.[48,49,50] One of these acids, 2-nitroso-1-naphthol-4-sulphonic acid, was found to give a green coloured complex with iron (Naphthol green G). Sarver[51] reported that 2-nitroso-1-naphthol-4-sulphonic acid formed complexes with ferrous and ferric iron, and he recommended the reagent for the spot-test detection of iron, cobalt, nickel and copper. Its use as a chromatographic spray reagent for a number of cations, including iron, has recently been described.[34] The reagent has been used in the spectrophotometric determination of cobalt,[52] and two complexes are reported to be formed with nickel, depending upon the pH of the solution.[53] No reference to work on the spectrophotometric determination of iron with this reagent has been found.

Cronheim,[54,55] while studying complex formation by *o*-nitrosophenol and its substituted derivatives, found that grass-green and brown complexes were formed with ferrous and ferric iron, respectively, but the formation of the ferric complex was not quantitative.

In the study to be described, both ferrous and ferric iron gave deeply coloured complexes with 2-nitroso-1-naphthol-4-sulphonic acid, in confirmation of Sarver's observation, but the intensity of the colour with ferric iron was found to depend on the volume of complexing

agent present in excess of the theoretical requirement. Consequently, before the reagent was added, all iron was reduced to the ferrous state with hydroxylamine in acid solution. This produced no deterioration in sensitivity, and the procedure given later (p. 795) remains general for the spectrophotometric determination of iron. Further advantages of using the lower valency state complex for the determination are that (*i*) the ferrous extract from the chromatogram does not require oxidation, while reduction to the ferrous state can be carried out in the cold, and (*ii*) the reducing "atmosphere" of the solution ensures the stability of the reagent.

In the following sub-sections are included details of the reaction between 2-nitroso-1-naphthol-4-sulphonic acid and both ferric and ferrous iron, and the effects on the recommended procedure of pH, reagent concentration, the nature of the anion, concentration of the reducing agent, time of colour development and the iron concentration at the stage of determination. The molecular ratio of complexing agent to ferrous iron has also been determined by two methods.

EXPERIMENTAL—

All optical-density measurements were made with a Unicam SP500 spectrophotometer, with 10-mm glass cells fitted with lids.

SOLUTIONS USED—

Standard solution of ferric nitrate (100 *μg of iron per ml*)—Pure iron wire was dissolved in dilute nitric acid by warming, and the resulting solution was filtered and standardised gravimetrically by precipitation of the hydroxide and ignition to ferric oxide. An appropriate aliquot of this solution was diluted to 1 litre in a calibrated flask.

Standard solution of ammonium ferric sulphate (100 *μg of iron per ml*)—0·8634 g of AnalaR ammonium ferric sulphate, $(NH_4)_2SO_4.Fe_2(SO_4)_3.24H_2O$, was dissolved and made up to 1 litre in water containing 1 ml of concentrated sulphuric acid, sp.gr. 1·84.

Hydroxylamine hydrochloride—A 5 per cent. w/v aqueous solution.

Acetic acid, 0·20 M.

Sodium acetate solution, 0·20 M.

Sodium hydroxide solution, 2 M.

Complexing reagent—0·247 g of sodium 2-nitroso-1-naphthol-4-sulphonate was dissolved in a mixture of methanol and water (1 + 4) and made up to 100 ml in a calibrated flask. A very slight brown residue remained, which was filtered off in a Jena No. 3 sintered-glass crucible.

EFFECT OF pH ON REAGENT AND COMPLEX—

With a 1·0-ml pipette, 1·0 ml of complexing reagent was transferred to a 25-ml calibrated flask and 10·0 ml of buffer were added to produce the required pH; at pH 4, 4·5, 5·0 and 5·5, the volumes of 0·20 *M* acetic acid and 0·20 *M* sodium acetate solution added were 8·0 and 2·0, 6·0 and 4·0, 3·0 and 7·0, and 1·0 and 9·0, respectively. The mixture was diluted to 25 ml with distilled water and the optical density was measured against a blank of distilled water. The optical density at peak absorption rose sharply with increase in pH and so, for buffer solutions at pH greater than 7 (Table III), only 0·5 ml of the reagent solution was added.

TABLE III

BUFFER SOLUTIONS FOR PRODUCING pH VALUES GREATER THAN 7

pH	Buffer
7·4	9·0 ml of 0·20 *M* boric acid and 1·0 ml of 0·05 *M* sodium tetraborate solution
9·5	10·0 ml of a solution containing 20·0 g of sodium hydrogen carbonate and 10·0 g of anhydrous sodium carbonate per litre
11·0	10·0 ml of a 3 + 7 mixture of AnalaR ammonia solution, sp. gr. 0·880, and water

The results are shown in Fig. 9. Maximum optical density occurs at 425 ± 5 mμ, and the absorption at this wavelength rises to a maximum at approximately pH 8. The optical density is almost zero after 520 mμ, so that the colour of the excess of reagent has very little effect on the determination, since maximum absorption of the complex occurs at 700 mμ.

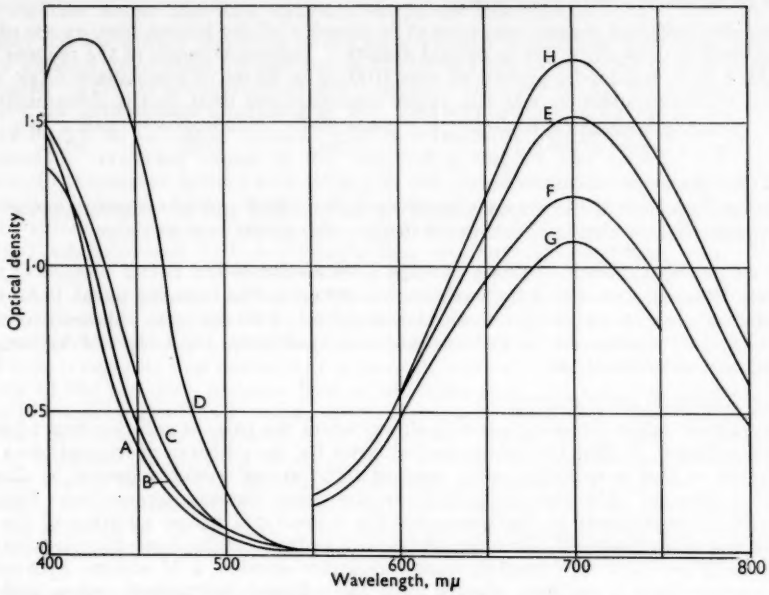

Fig. 9. Absorption spectra of reagent and of iron complex at various pH values: curves A, B, C and D, reagent at pH 4·0, 5·0, 5·5 and 9·5, respectively; curves E, F and G, 40 μg of iron, as nitrate, at pH 5·0, 9·5 and 11, respectively; curve H, 40 μg of iron, as sulphate, at pH 5·0

VARIATION OF ABSORPTION SPECTRA OF THE FERROUS COMPLEX WITH VARIATION IN pH—

A 1·0-ml portion of standard ferric nitrate solution, 2·0 ml of 5 per cent. hydroxylamine hydrochloride solution, 1·0 ml of complexing reagent solution and 10 ml of buffer were diluted to 25 ml in a calibrated flask with distilled water. The components were added in that order and the final solution was deep green; the buffer solutions used are listed above. The optical densities of the solutions were measured at various wavelengths against a similarly prepared reagent blank. The maximum optical density occurred at 700 ± 10 mμ, and at pH 5·0 ± 0·5 (Fig. 9).

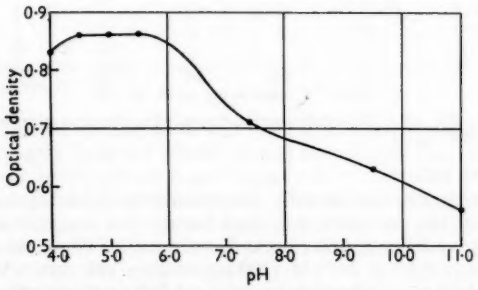

Fig. 10. Variation of peak height with change in pH

The peak height at 700 mμ was then plotted against pH for a concentration of 2 μg of iron per ml, standard ammonium ferric sulphate solution and the same procedure being used (Fig. 10). Since pH 5 occurs at approximately the centre of the portion of the curve with the least slope, all further work was carried out at this pH.

EFFECT OF REAGENT CONCENTRATION—

Provided sufficient reagent was present to complex all the ferrous iron, excess of reagent produced only a very slight rise in optical density. Various volumes of the reagent solution were added to a standard quantity of iron (100 μg in 25 ml of ammonium ferric sulphate solution); this concentration was the upper concentration limit in the determinations.

Molar concentration of reagent ..	$3{\cdot}6 \times 10^{-4}$	$7{\cdot}2 \times 10^{-4}$	$10{\cdot}8 \times 10^{-4}$
Optical density at 700 mμ	1·720	1·725	1·725

EFFECT OF TIME OF DEVELOPMENT—

The development of the colour formed by ferrous iron and the reagent appeared to be instantaneous and no alteration in optical density was noted over 24 hours.

EFFECT OF HYDROXYLAMINE HYDROCHLORIDE CONCENTRATION—

Little effect was produced by a reasonable excess of the reducing agent (5 ml of 5 per cent. solution), but at very high concentrations (10 ml of 20 per cent. solution) results were low. With the volume used in the recommended procedure, the excess of hydroxylamine produced no noticeable effect.

ORDER OF ADDITION OF REAGENTS—

The rate of colour development was slower when the reagent solution was added after the buffer solution, so that the recommended order for the addition of reagents to a weakly acid solution of iron is reducing agent, reagent solution and buffer, followed by dilution to the correct volume. The iron extracts from the paper chromatograms were highly acid and had to be neutralised to approximately the correct pH before addition of the buffer. Recovery was quantitative if, after the addition of hydroxylamine hyrochloride solution and a drop of 0·05 per cent. w/v methyl orange indicator solution, 2 *M* sodium hydroxide was added dropwise from a capillary pipette until the solution just turned yellow (pH 5).

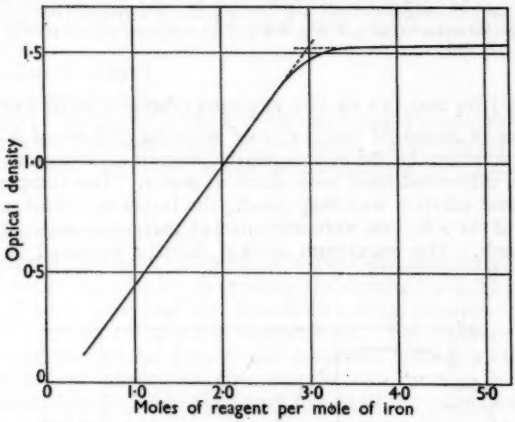

Fig. 11. Mole-ratio method applied to the iron complex at 700 mμ

STRUCTURE OF THE COMPLEX—

The nature of the complex was initially determined by the mole ratio method of Yoe and Jones.[37] In this method, the variation of optical density at a constant wavelength is observed for a series of solutions containing different molecular ratios of metal to reagent. The iron concentration chosen was $7{\cdot}17 \times 10^{-5}$ *M* (100 μg of iron per ml). Various volumes of 2-nitroso-1-naphthol-4-sulphonic acid solution were added to the iron solution, by means of an Agla micrometer syringe, to cover the range of ratios 0 to 5 moles of reagent per mole of iron. The optical densities of these solutions were measured at 700 mμ against a similarly prepared reagent blank. The results are shown in Fig. 11, which indicates that in the complex 1 mole of iron combines with 3 moles of reagent. The same result was obtained by the slope-ratio method.[56]

EFFECT OF THE ANION AND THE VALENCY STATE OF THE IRON—

When the absorption spectrum of the complex was determined with ammonium ferric sulphate solution (containing 100 μg iron in 25 ml) and the colorimetric procedure described earlier under "Effect of pH on reagent and complex," the peak height was appreciably above that found for ferric nitrate solution (Fig. 9). This was thought to be due to the presence of excess of nitrate in the latter solution and its subsequent interference in the colorimetric determination. Provided excess of the complexing reagent was present, the absorption spectrum of the complex formed with ferric iron was the same as that obtained with a similar concentration of ferrous iron. When sufficient complexing reagent for a 1 to 3 complex was added to ammonium ferric sulphate, the optical density of the mixture did not reach the value that was produced with a corresponding concentration of ferrous iron. When the amount of reagent added was sufficient for a ratio of 1 to 4, full colour developed in about 10 minutes. At 1 to 5 or lower ratios, the colour developed almost immediately.

Since excess of complexing reagent gives complexes with both ammonium ferrous and ammonium ferric sulphates at pH 5 that have identical absorption spectra at similar concentrations of iron, it suggests that the valency state of the central metal atom is the same. During the study of the reactions between ferrous and ferric iron and *iso*nitrosodimethyldihydroresorcinol (*iso*nitrosodimedone), similar observations were made by Shome.[57,58] Cronheim[54,55] found that *o*-nitrosophenol reduced ferric to ferrous iron in strong light. Because of the excess of 2-nitroso-1-naphthol-4-sulphonic acid required for full colour development with ferric iron, it may be that this oxidation - reduction reaction occurs here also. In the determinations of ferric iron described later, hydroxylamine was added to the solution immediately before the addition of the reagent. This minimised the excess of reagent required for full colour development and hence reduced the blank reading.

PREPARATION OF CALIBRATION GRAPH—

Various volumes of the ammonium ferric sulphate solution were transferred to 25-ml calibrated flasks, so that they contained between 0·0 and 4·0 μg of iron per ml. To each was added 2·0 ml of 5 per cent. hydroxylamine hydrochloride solution, 1·0 ml of complexing reagent solution, 3·0 ml of 0·20 *M* acetic acid and 7·0 ml of 0·20 *M* sodium acetate solution. The solutions were made up to volume, and their optical densities were measured at 700 mμ against a similarly prepared reagent blank.

Beer's law was obeyed over the range studied, and the slope of the calibration graph was 0·428 optical-density units per μg of iron per ml. Ten determinations on 4·0 μg of iron per ml and 0·50 μg of iron per ml gave an average of 3·98 μg and 0·50 μg of iron per ml, respectively. On comparing the sensitivities of the reactions of 2-nitroso-1-naphthol-4-sulphonic acid, nitroso-R salt, 1 : 10-phenanthroline and disodium catechol-3 : 5-disulphonate by the method of Woods and Mellon,[59] we have—

	Nitroso-R salt	2-Nitroso-1-naphthol-4-sulphonic acid	1 : 10-Phenanthroline	Disodium catechol-3 : 5-disulphonate
Iron required for 50 per cent. transmission for 1-cm cells, using maximum absorption of curve, p.p.m.	0·67	0·71	1·7	2·9

While not quite so sensitive as nitroso-R salt, 2-nitroso-1-naphthol-4-sulphonic acid has the advantages that the reagent does not absorb at the wavelength used for the determination of the iron and that colour development is instantaneous.

Since the chromatographic separation gave pure iron solutions, a study of the effects of other ions on the reagent was not undertaken. A short spectrophotometric study of the reaction between the complexing reagent and cupric copper was carried out, but the slope of the calibration graph under the optimum conditions for absorption was only 0·181 optical-density units per μg of copper per ml.

PROCEDURE

At least two chromatographic separations were carried out on each of the following mixtures: (*a*) 0·025 ml of the 1 to 1 solution described in Table II on a double-strip chromatogram (*i.e.*, 25 μg of each valency state); (*b*) 0·00445 ml (2 spots from the 2·225-μl capillary

dropper) of the 1 + 1 solution used for the investigation with the capillary dropper (see p. 789) on a triple-strip chromatogram (27·8 μg of each valency state); (*c*) 0·05 ml of the 1 to 1 solution described in Table II on a single-strip chromatogram (50 μg of each valency state) and (*d*) 0·1 ml of all solutions described in Table II (except that 0·2 ml of the 1 to 49 solution was used) on single-strip chromatograms.

All volumes of 0·025 ml or greater were placed on the chromatograms from an Agla micrometer syringe. Volumes of 0·1 ml were placed on the starting line in two applications of 0·05 ml. The first band was allowed to dry for 10 minutes before the second was applied. It was found that the R_F for ferrous iron increased and the R_F for ferric iron decreased with the time of drying of the second band. The importance of time of drying in quantitative work has also been stressed by Lacourt, Sommereyns, Stadler-Denis and Wantier in the separation of chromium and molybdenum.[60] The minimum times of drying necessary for complete separation, shown in Table V, were found by experience and are only intended as a rough guide. An upper limit to the time of drying was also found to exist owing to the decreased stability of each valency state on dry paper. This limit was not critical except at extreme ratios of ferrous to ferric iron.

On the double-strip chromatograms (25 μg of each valency state), 0·025 ml of the mixed solution was placed on the starting line in two applications of equal volume; each band was allowed to dry for 3 to 4 minutes. On the triple-strip chromatograms, the first spot from the capillary dropper was dried for 2 to 3 minutes and the second spot for 3 to 4 minutes.

At the ratio 1 to 49, 0·2 ml of solution was applied to the starting line in three applications: 0·08 ml, then 13 minutes' drying; 0·08 ml, then 10 minutes' drying; and finally 0·04 ml. Elution was begun 15 minutes after this final application.

The separations were usually carried out in batches of about eight with one blank chromatogram. If possible, each chromatogram in a batch was cut from the same sheet of acid-washed paper. Lengths of paper 6·5 cm wide, but between only 20 and 30 cm long, could still be used after being joined with adhesive tape to unwashed paper.

The chromatograms were placed in the ascending-elution apparatus[21] and removed after a length of run of between 10 and 18 cm. The most suitable lengths of run, as found by experience, are shown in Table V. Eluted chromatograms were immediately held in a gas-jar charged with a few millilitres of ammonia solution, sp.gr. 0·880. This caused the iron to be deposited as hydroxide; ferrous hydroxide rapidly oxidised to ferric hydroxide.

The positions of the iron bands were marked in ultra-violet illumination (see Fig. 6). In any one batch in which similar or slightly differing ratios were being investigated, the sizes of the iron bands were measured and, for each valency state, a similarly sized strip of paper was cut from the quantitative chromatogram and from the blank with a new pair of heavily plated scissors.

The excised bands were cut into 4 or 6 pieces so as to fit easily into 100-ml beakers. These beakers, covered with watch-glasses, were then ready for the acid extraction.

CHROMATOGRAMS OF IRON WITH OTHER METALS—

The general procedure was as for the valency-state separations. Two 0·05-ml aliquots of the mixed solution were applied to the chromatogram to give a total of 0·1 ml, each band being allowed to dry for 10 minutes. The length of run each time was about 15 cm in 90 minutes. After the eluted chromatogram had been held in ammonia vapour, the dark bands of chromium, iron, cobalt and copper showed up clearly in ultra-violet light. With each metal, a band of paper 6·5 × 5·0 cm cut from the chromatogram was sufficient to remove the iron for determination. After removal of iron, the nickel was detected by a rubeanic acid spray. The usual reagents for manganese, *e.g.*, oxine, are not very satisfactory, so a new spray reagent was sought. The best found so far consisted of 4 per cent. v/v of salicylaldehyde in 1 + 1 aqueous ethanol. After the spraying, the paper was held in ammonia. The band for manganese appeared brown on a yellow background, but was clearer in ultra-violet illumination as dark brown on a brightly fluorescent blue-green background. Other metals (about 10 μg in a 7-mm spot) that gave reactions differing from that of ammonia alone were copper (yellow-green), silver (light brown), lead and nickel (yellow), vanadium as V^{IV} or V^{V} and manganese (grey-brown), chromium (faint yellow-green), ferrous and ferric iron (different shades of brown) and cobalt (pale yellow-brown). In ultra-violet illumination all these metals except silver and chromium appeared as dark spots on the fluorescent background.

TABLE IV

RESULTS OF DETERMINATIONS ON A SINGLE BATCH OF ACID-WASHED PAPER

Ratio of Fe^{II} to Fe^{III}	Iron present as		Iron found as						Mean errors	
	Fe^{II}, μg	Fe^{III}, μg	Fe^{II}, μg	Fe^{III}, μg	Fe^{II}, μg	Fe^{III}, μg	Fe^{II}, μg	Fe^{III}, μg	Fe^{II}, %	Fe^{III}, %
1 to 1	25*	25*	24·6	24·8	24·6	24·3	—	—	1·6	1·8
	27·8†	27·8†	27·5	28·0	27·7	28·3	—	—	0·72	1·3
	50	50	50·8	49·5	51·0	49·9	49·9	49·2	1·3	0·93
	100	100	99·0	99·8	99·4	100·3	99·5	99·3	0·70	0·40
	500	500	499·0	501·5	497·0	497·0	504·0	504·0	0·53	0·57
1 to 5	100	500	100·5	494·0	101·0	497·0	101·2	490·0	0·90	1·3
1 to 10	100	1000	99·4	1002·0	100·6	1005·0	99·3	995·0	0·63	0·40
1 to 24	50	1200	49·5	1217·0	49·7	1221·0	51·0	1225·0	1·2	1·8
1 to 49	50	2450	49·7	2401·0	51·0	2440·0	—	—	1·3	1·2
5 to 1	500	100	512·0	101·0	505·0	101·0	495·0	101·0	1·5	1·0
10 to 1	1000	100	994·0	101·4	982·0	103·0	997·0	101·8	0·90	2·1
25 to 1	1250	50	1238·0	51·0	1238·0	51·5	1230·0	51·8	1·2	2·9

* Double-strip method.
† Using a capillary dropper and the triple-strip method.

When analyses of unknowns are carried out, the ferrous to ferric ratio is often known approximately, and Table V suggests experimental conditions that will result in a satisfactory separation.

TABLE V

RECOMMENDED EXPERIMENTAL CONDITIONS FOR VARIOUS RATIOS OF Fe^{II} TO Fe^{III}

	Amount of iron, μg	Ratio	Minimum time of drying of applied band for separation, minutes	Minimum length of run, cm	Maximum R_F of leading edge of Fe^{II}*	Minimum size of band to be cut out,† cm
Iron as Fe^{II}	25	1 to 1	2	10	0·40	5·0
	50	1 to 1	6	15	0·35	6·0
		1 to 24	6	17	0·35	6·0
		1 to 49	10 (0·2 ml)	18	0·40	6·5
	100	1 to 1	6	15	0·40	6·5
		1 to 5	6	16	0·40	6·5
		1 to 10	6	16	0·40	6·5
	500	1 to 1	8	17	0·40	6·5
		5 to 1	8	17	0·45	7·0
	1000	10 to 1	8	17	0·40	7·0
	1250	25 to 1	8	17	0·45	7·5
					Minimum R_F of rear of Fe^{III}	
Iron as Fe^{III}	25	1 to 1	2	10	0·55	3·0
	50	1 to 1	6	15	0·65	3·0
		25 to 1	8	17	0·50	5·5
	100	1 to 1	6	15	0·55	4·5
		5 to 1	8	17	0·50	5·0
		10 to 1	8	17	0·50	6·0
	500	1 to 1	8	17	0·45	7·0
		1 to 5	6	16	0·45	5·0
	1000	1 to 10	6	16	0·45	8·5
	1200	1 to 24	6	17	0·40	8·5
	2450	1 to 49	10 (0·2 ml)	18	0·40	10·5

* At the recommended minimum time of drying and length of run.
† In all cases (except 25 μg to 25 μg, where width of paper is 3 cm), the width of the paper is 6·5 cm, and so the area of paper cut out is 6·5 times the value given in this column. Except in a few instances (as shown in Table II), the volume of solution applied to the starting line was 0·1 ml.

EXTRACTION—

To each iron-bearing section of the chromatogram was added 10 ml of 50 per cent. v/v AnalaR hydrochloric acid, and the solution was boiled for 1 minute on an electric hot-plate. This extract was poured into another beaker; the extraction was repeated with 10 ml of 25 per cent. v/v hydrochloric acid, followed by 10 ml of distilled water. The three extracts were combined, filtered through a Jena No. 3 sintered-glass funnel and evaporated to a few drops. It was then ready for spectrophotometric determination.

SPECTROPHOTOMETRIC DETERMINATION OF THE EXTRACTS—

The few drops of solution were transferred to a 25-ml calibrated flask, together with the washings, and 2·0 ml of 5 per cent. hydroxylamine hydrochloride solution were added. Then 1 drop of 0·05 per cent. w/v aqueous methyl orange was added and 2 *M* sodium hydroxide was run in dropwise from a capillary pipette until the indicator turned yellow. Finally, 1·0 ml of 0·247 per cent. w/v 2-nitroso-1-naphthol-4-sulphonic acid in 1 + 4 methanol - water mixture, 3·0 ml of 0·20 *M* acetic acid and 7·0 ml of 0·20 *M* aqueous sodium acetate solution were added. The solution was made up to volume and the optical density was measured at 700 mμ against a similarly prepared chromatogram blank.

RESULTS

Table IV shows the results obtained on a single batch of acid-washed paper. The errors tabulated are an indication of the total error from the whole method—the solutions, the elution, impurities in the paper and reagents, removal of the iron, the extraction and colorimetric determination.

One blank was run with each batch of chromatograms, and Table VI gives the blank readings in the ferrous and ferric positions for ten consecutive sets of runs. For the first five

TABLE VI

BLANK READINGS ON ACID-WASHED WHATMAN NO. 1 FILTER-PAPER

Width of strip, cm	Fe^{II} Area of paper cut out, sq. cm	Fe^{II} Total iron in blank, μg	Fe^{III} Area of paper cut out, sq. cm	Fe^{III} Total iron in blank, μg
2	5·4	3·9	6·0	0·76
3·1	17·05	5·8	9·3	5·6
	13·95	3·7	10·85	5·7
	14·55	4·8	9·3	4·8
	7·75	4·9	7·75	2·5
6·5	47·5	1·2	32·5	2·6
	47·5	2·0	32·5	3·2
	45·5	4·9	39·0	2·9
	39·0	0·88	45·5	1·1
	45·5	3·7	32·5	3·1

TABLE VII

DETERMINATION ON OTHER TYPES OF PAPER

Whatman filter-paper No.	Length of run, cm	Time of elution	Ferrous iron Fe^{II} found, μg	Error, %	Blank, μg	Width of band,* cm	Blank reading, μg per sq. cm	Ferric iron Fe^{III} found, μg	Error, %	Blank, μg	Width of band,* cm	Blank reading, μg per sq. cm
1	16·2	2 h. 30 m.	240·0	4·0	23·0	4·1	0·86	232·5	7·0	28·0	4·2	1·03
54	16·1	2 h. 30 m.	242·5	3·0	14·8	5·0	0·46	248·5	0·6	16·4	3·7	0·68
540	17·6	2 h. 55 m.	248·5	0·6	6·7	5·0	0·22	247·5	1·0	8·9	4·4	0·31

* Width of the band cut from the paper after elution. The "length" of the band is 6·5 cm (*i.e.*, the "width" of the chromatogram is 6·5 cm).

runs (top five lines of results), the average is 0·39 and 0·45 μg of iron per sq. cm in the ferrous and ferric bands, respectively, and the second five runs average 0·062 and 0·071 μg of iron per sq. cm. These blank readings include the blank of the colorimetric method and so, when

expressed in μg of iron per sq. cm of paper, the values decrease with increasing area of paper cut from the chromatogram.

The work described in Part I had indicated that Whatman Nos. 54 and 540 filter-papers were the most free from iron, and so a separation of 250 μg of each valency state was carried out and a comparison made with Whatman No. 1 filter-paper. The volume of solution applied to the starting line was 0·05 ml and the time of drying of the band was about 6 minutes (band appeared just damp). A blank was run with each type of paper.

From the results in Table VII it can be seen that, when an accuracy of within 1 or 2 per cent. is acceptable, Whatman No. 540 filter-paper is satisfactory for the separation of about 250 μg of each valency state.

SEPARATION OF 100 μg OF FERRIC IRON FROM 1 mg OF ANOTHER METAL—

Table VIII shows the amount of iron found by this procedure in mixtures of 100 μg of ferric iron with 1 mg of chromium, manganese, cobalt, nickel or copper. Also shown are the approximate R_F value for the leading edge of the second metal and the R_F separation between this leading edge and the rear edge of the ferric iron band.

TABLE VIII

SEPARATION OF IRON AND OTHER METALS

Second metal	Fe^{III} found (100 μg taken), μg	R_F of leading edge of second metal ($\pm$ 0·025)	R_F separation between second metal and iron
Chromium	100·0, 100·6	0·25	0·20
Manganese	99·0, 100·3	0·375	0·075
Cobalt	99·5, 100·2	0·275	0·175
Nickel	100·5, 100·3	0·25	0·20
Copper	100·0, 100·4	0·425	0·025

CONCLUSION

By using a simple ascending-elution technique, it was found possible to determine both ferrous and ferric iron in ratios between 1 to 49 and 25 to 1. The method showed maximum accuracy at ratios close to 1 to 1 and 100 to 500 μg of each valency state, and for a single application of 0·05 ml of the unknown solution to a 6·5-cm wide chromatogram.

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NOTE—Reference 34 is to Part II of this series.

DEPARTMENT OF INORGANIC AND PHYSICAL CHEMISTRY
THE UNIVERSITY
BRISTOL, 8

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The Extraction and Absorptiometric Determination of Uranium as Thiocyanate

BY J. CLINCH* AND MARGARET J. GUY

A method is proposed for the determination of uranium in the presence of many other elements. The uranium is extracted as thiocyanate from a solution containing ethylenediaminetetra-acetic acid at a pH of 3·5 to 3·9 into 32·5 per cent. v/v tributyl phosphate in carbon tetrachloride. The optical density of the dried organic extract is compared with that of extracts containing no uranium at 350 mμ in a spectrophotometer. The method has a coefficient of variation of about 0·6 per cent. Application of the method to the determination of uranium in low-grade ores and to the determination of uranium in thorium oxide are given.

BECAUSE of a need for the determination of uranium in thorium-containing materials, an examination has been made of the various methods available for the determination of microgram amounts of uranium. Although several sensitive methods are available, they generally demand that the uranium should be in as pure a state as possible for determination. The most sensitive method depends on the fluorescence of uranium in fused sodium fluoride melts.[1] This method gives errors of about ± 10 per cent. The uranium must, however, be separated from most other elements, because of their quenching effect. Apparatus to measure the

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fluorescence is also required; without the apparatus, and with use of visual standards, errors are somewhat greater than 10 per cent. Polarographic methods also require purification of the uranium.[2]

Several colorimetric methods have been investigated. The oxine method of Silverman, Moudy and Hawley[3] requires rigid pH control and even in the presence of ethylenediaminetetra-acetic acid (EDTA), which is added to suppress the interference of elements such as iron, high and variable blanks have been found. Although the diethyldithiocarbamate method of Jones[4] includes a step in which the uranium is purified by making use of the insolubility of uranium diethyldithiocarbamate in carbon tetrachloride, it is still unable to deal with more than small amounts of interfering elements.

The aqueous thiocyanate method of Currah and Beamish[5] has often proved to be the most useful colorimetric method, despite the low sensitivity. Ferric iron is reduced to the non-interfering ferrous state with stannous chloride, but molybdenum and rhenium are major interfering elements. Because of the acid conditions of the method, thorium can be tolerated, even in large amounts. An objection to the method in practice has been the production of a spurious yellow colour on the addition of stannous chloride to the sample.

The method now proposed was suggested by the observations of Freiser and his associates,[6,7] who found that ferric and cupric thiocyanates could be extracted into tributyl phosphate or mixtures of tributyl phosphate and carbon tetrachloride. Experiments showed that uranium thiocyanate could also be extracted into mixtures of tributyl phosphate and carbon tetrachloride to give, depending on the amount of uranium present, colourless to yellow solutions. To prevent the extraction of ferric thiocyanate, a solution of EDTA was added to the aqueous layer before extraction. Uranium was still found to be extracted, but ferric ion was retained in the aqueous layer.

Experimental

Wavelength of absorption—

Twenty-five millilitres of a solution of uranium nitrate containing 0·01364 g of uranium per litre were mixed with 5 g of ammonium thiocyanate and 1 g of the disodium salt of EDTA. The solution was diluted to 50 ml in a 100-ml graduated separating funnel and 10 ml of a 25 per cent. v/v solution of tributyl phosphate in carbon tetrachloride were added. After the mixture had been shaken for 30 seconds, the layers were allowed to separate and the lower organic layer was withdrawn into a 25-ml flask containing about 0·5 g of anhydrous sodium sulphate. After the organic layer had been set aside for 5 minutes to allow it to dry, its optical densities were compared at wavelengths from 300 to 380 mμ with a solution prepared in a similar fashion, but with the uranium omitted. Graphs of the same form as those shown in Fig. 1 were obtained. A notable feature of the absorption was the peak at 350 mμ instead of the expected gradual increase in absorption as the wavelength decreases, a feature characteristic of the aqueous thiocyanate method.[5] Subsequent measurements were always made at 350 mμ. Because of the width of the absorption peak, it was found possible to increase the slit width used in these experiments from 0·22 mm (corresponding to a 0·5-mμ band pass) to 0·4 mm, thereby increasing the sensitivity of the readings. The previous work of Freiser and his associates suggested that the extraction might depend on the ratio of tributyl phosphate to carbon tetrachloride, the pH of the extracted solution and the concentration of thiocyanate.

Ratio of tributyl phosphate to carbon tetrachloride—

Ten millilitres of organic solvent were used and 0·341 mg of uranium was extracted from 50 ml of a solution containing 5 g of ammonium thiocyanate and 0·5 g of the disodium salt of EDTA. The results are shown in Fig. 2.

The maximum absorption appeared to be reached at a concentration of 32·5 per cent. v/v of tributyl phosphate in carbon tetrachloride; a solvent having this composition was used in subsequent work.

Concentration of ammonium thiocyanate—

Ten millilitres of 32·5 per cent. v/v tributyl phosphate in carbon tetrachloride were used and 0·341 mg of uranium was extracted from 50 ml of solution containing 0·5 g of the disodium salt of EDTA and various volumes of 50 per cent. v/v ammonium thiocyanate solution. The results are shown in Fig. 3.

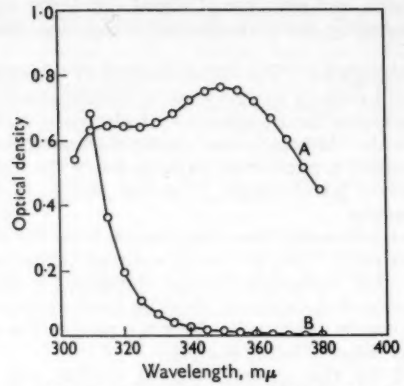

Fig. 1. Variation of optical density with wavelength: curve A, solution containing 0·341 mg of uranium measured against blank solution; curve B, blank solution containing no uranium measured against carbon tetrachloride. A 32·5 per cent. v/v mixture of tributyl phosphate in carbon tetrachloride was used as the solvent for the preparation of both curves

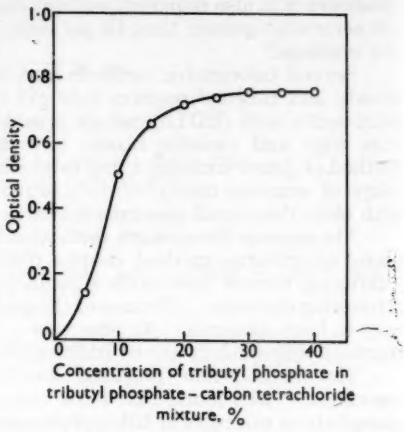

Fig. 2. Variation of optical density of a solution containing 0·341 mg of uranium with percentage of tributyl phosphate in tributyl phosphate - carbon tetrachloride mixture

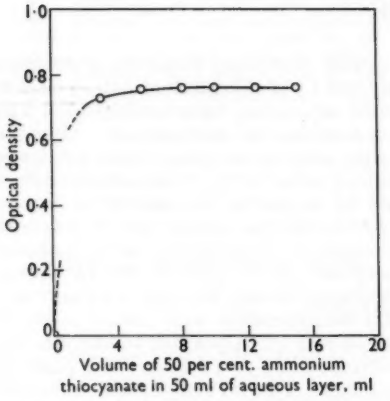

Fig. 3. Variation of optical density of a solution containing 0·341 mg of uranium with concentration of ammonium thiocyanate in the aqueous layer

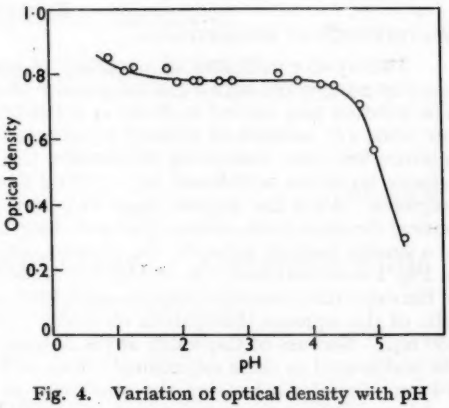

Fig. 4. Variation of optical density with pH

The results plotted show that the optical density increases to a maximum with increasing thiocyanate concentration. This is an advantage over previous uranium thiocyanate methods, in which the optical density always increases with the thiocyanate concentration and no maximum is attained. Hence, 10 ml of 50 per cent. w/v ammonium thiocyanate solution were used in subsequent work.

EFFECT OF pH—

If the pH of the aqueous layer is between 1·9 and 3·9, the optical density of the extract is constant (Fig. 4). Above pH values of 3·9 the extraction of uranium rapidly diminishes, and on shaking solutions of pH values of less than 1·9 the organic layer becomes pink and its optical density higher. Tests with various interfering elements (*vide infra*) showed that interference was often most marked at pH values of less than 3. For this reason we have used

the narrower pH range of 3·5 to 3·9, in which there is very little interference from those cations that react with thiocyanate.

The organic extract may be kept for several hours without change if prepared in the correct pH range. The solution should be kept away from ground-glass surfaces, since it readily extracts traces of iron.

Method

Apparatus—

Optical-density measurements were made on a Uvispek spectrophotometer. A Cambridge Instrument Co. bench-type direct-reading pH meter was used for the pH measurements.

Reagents—

n-*Tributyl phosphate*—Wash with 10 per cent. w/v sodium carbonate solution and redistil under reduced pressure (30 mm of mercury) to remove water, butanol and mono- and dibutyl phosphates.

Carbon tetrachloride.

Tributyl phosphate in carbon tetrachloride, 32·5 per cent. v/v—Dilute 325 ml of tributyl phosphate to 1 litre with carbon tetrachloride.

Ammonium thiocyanate solution, 50 per cent. w/v—Dissolve the contents of a 500-g bottle of ammonium thiocyanate in warm water, filter through a paper-pulp pad and dilute to 1 litre with water. Care should be taken over the selection of a suitable grade of ammonium thiocyanate; inferior grades turn yellow and a precipitate forms in the solution within a few days. The AnalaR grade has proved satisfactory.

EDTA solution, 10 per cent. w/v—Dissolve 100 g of the disodium salt of ethylenediaminetetra-acetic acid in 900 ml of boiling water, filter, cool and dilute to 1 litre.

Ammonia solution, sp.gr. 0·880.

Ammonia solution, M.

Hydrochloric acid, 11·4 M.

Hydrochloric acid, M.

Procedure—

The uranium may be in nitrate, chloride, sulphate or perchlorate solution. Add 20 ml of 10 per cent. w/v EDTA solution and adjust the pH to between 3·5 and 3·9 with *M* ammonia solution or *M* hydrochloric acid. Add 10 ml of 50 per cent. w/v ammonium thiocyanate solution, mix, re-check the pH and transfer the solution to a 100-ml graduated separating funnel. Dilute to 50 ml with water and add 10·0 ml of 32·5 per cent. v/v tributyl phosphate in carbon tetrachloride. Shake the separating funnel vigorously for 30 seconds, allow the organic layer to separate and run it off into a small flask containing about 0·5 g of anhydrous sodium sulphate. Dry the organic layer by swirling the contents of the flask.

Measure the optical density of the solution in 1-cm cells against a blank prepared in a similar fashion, at a wavelength of 350 mμ and with a slit width of 0·4 mm.

Results

Extraction of uranium into the organic layer appears to be extremely rapid, a 5-second shaking period being sufficient for pure uranium solutions. A 30-second shaking period was adopted. Temperature effects were not investigated. The optical density obeys Beer's law up to a value of 1·5 (the maximum tested); the molecular extinction coefficient is 5310 and the precision (σ) is 1·7 μg of uranium. The molecular extinction coefficient compares favourably with the values by previous published methods—

	Molecular extinction coefficient
Peroxide (depending on method)[8]	1000 to 1500
Diethyldithiocarbamate[4]	4650
Oxine (depending on wavelength)[3]	3640 to 6550
Dibenzoylmethane[9]	21,000
Aqueous thiocyanate[5]	3440
Aqueous acetone - thiocyanate[10]	3850

Since many metallic ions give colours or precipitates with thiocyanate ion in water, an extensive survey was undertaken to determine their effect on the method. Besides the well

known colours given by ferric iron, rhenium, molybdenum and cobalt ions, lead and nickel thiocyanates absorb in the ultra-violet region. Experiments were therefore performed with solutions of various pH values from 2·5 to 3·9 containing 50 mg of metal. It was generally found that interference, if any, was much less at the higher pH value. More experiments were therefore made at pH values of about 3·8, with 20, 50, 100 and 200 mg of metal being used. The following metallic ions in the given valency states were found to give no interference at all at pH 3·8 and with up to 200 mg of metal: Tl^{I}, Ag^{I}, Cu^{II}, Ni^{II}, Mn^{II}, Cd^{II}, Pb^{II}, Sn^{II}, Bi^{III}, Fe^{III}, Cr^{III}, Sb^{III}, light Ln^{III}, (heavy Ln + Y)^{III}, Sc^{III} (60 mg was the most tried), Al^{III}, Ti^{IV} (100 mg was the most tried), Th^{IV}, V^{V} (80 mg was the most tried) and Re^{VII}.

Elements that have caused difficulties are—

(*i*) *Molybdenum and tungsten*—Molybdenum (as ammonium molybdate) interferes considerably at pH values less than 3·6. At a pH of 3·85, 700 mg of molybdenum gave an optical density of 0·032 in the absence of uranium. In the presence of uranium, molybdenum causes considerable diminution of the optical density. The effect is shown by the following values found in the presence of 0·341 mg of uranium at a pH of 3·8—

Molybdenum taken, mg	0	14	70	140	350
Optical density at 350 mμ	0·762	0·713	0·669	0·582	0·396

Tungsten (as ammonium tungstate) interferes similarly, but the effect is less pronounced, as shown by the following results, 0·341 mg of uranium again being present—

Tungsten taken, mg	0	20	50	200
Optical density at 350 mμ	0·764	0·742	0·708	0·582

Molybdenum should therefore be absent, but tungsten may be tolerated in amounts up to 10 mg.

(*ii*) *Cobalt*—Although cobalt appears to be largely complexed by EDTA, some extraction does occur and, with increasing cobalt content, the organic layers become tinged with blue. The effect is shown by the optical densities at 350 mμ in the presence of 0·341 mg of uranium—

Cobalt taken, mg	0	20	50	100	200
Optical density at 350 mμ	0·761	0·779	0·805	0·822	0·876

Small amounts of cobalt may therefore be tolerated, but amounts larger than 10 mg should be avoided.

(*iii*) *Zirconium, tin*^{IV} *and cerium*^{IV}—These elements were precipitated in the presence of EDTA at pH values of 3·8, and some of the uranium appeared to be co-precipitated. The effect is shown by the optical densities of various solutions containing zirconium and 0·341 mg of uranium, as follows—

Zirconium taken, mg	0	20	50	100	200
Optical density at 350 mμ	0·762	0·585	0·663	0·552	0·513

Tin^{IV} and $cerium^{IV}$ interfere similarly, but by reducing them to tin^{II} and $cerium^{III}$, interference is avoided.

(*iv*) *Gold and platinum*—In tests, 50 mg of gold as chloroauric acid appeared to be completely extracted into the organic layer and to colour it a deep orange. The solution absorbed strongly at 350 mμ, 10 mg of gold giving an optical density of 0·229 in 1-cm cells. The solution does not appear to obey Beer's law and the absorption is too insensitive for the method to have any practical use in the determination of gold. The effect of gold can be completely suppressed by the addition of sufficient potassium cyanide almost to decolorise the solution.

Platinum (added as chloroplatinic acid) interferes similarly, but the interference cannot be completely eliminated with cyanide.

(*v*) *Mercury*—Mercurous and mercuric ions are extracted and absorb strongly in the presence and absence of uranium. Mercury ions must therefore be absent.

Anions that cause no interference in amounts up to 5 g added as the ammonium salts include chloride, nitrate, sulphate and perchlorate. The effects of other groups are considered below—

(*i*) *Nitrites and nitrogen dioxide*—These must be absent because of the formation of a yellow or red colour, which is extractable. Care should be exercised even when nitrates are used, since these may contain nitrogen dioxide. Pure neutral nitrates appear to be

quite safe; up to 10 g of ammonium nitrate has been used in an extraction without alteration of the final result. Small amounts of nitrogen dioxide can be eliminated by warming with 0·5 g of urea before extraction, but larger amounts are conveniently removed by heating to fumes with perchloric acid.

(*ii*) *Fluoride*—Interference with the extraction is found at very low levels, as shown by the following results, 0·341 mg of uranium being present—

Fluoride taken, mg	0	2	5	10	20
Optical density at 350 mμ	0·762	0·729	0·613	0·426	0·115

The fluoride can be eliminated by heating to fumes with perchloric acid with or without the addition of boric acid, or complexed by adding aluminium nitrate to the sample.

(*iii*) *Phosphate*—Uranium is precipitated by phosphate under the pH conditions used in the method. Precipitation is, however, slow and, if acid solutions are rapidly neutralised and extracted, the correct result is obtained. Uranium precipitated in the presence of phosphate may be brought into solution by adding acid. The rate of precipitation of the phosphate is diminished the lower the pH of the solution. Up to 200 mg of P_2O_5 have been satisfactorily dealt with in this way. Arsenate behaves similarly.

(*iv*) *Organic acids*—The effect of acetic, citric and tartaric acids has been examined. The absorption is diminished at amounts greater than about 1 g, but can be neglected for 0·5-g amounts. All the acids can be eliminated by heating to fumes with nitric and perchloric acids.

(*v*) *Dichromate and permanganate*—Dichromate ions give an extractable brown colour with thiocyanate, which interferes strongly. Reduction to the chromic state with sulphur dioxide removes the interference. Permanganate is reduced by EDTA and is non-interfering.

Applications of the method

The method has been successfully used for examining the distribution of uranium in a thorium process from the starting ore containing about 0·1 per cent. of U_3O_8 and 6 per cent. of ThO_2 down to the final pure thorium oxide containing less than 0·2 p.p.m. of U_3O_8. Purification from gross amounts of impurities is easily effected by using the standard methods involving extraction with ether, either continuous solvent extractors[11] or extraction from cellulose columns.[12] Two examples of these techniques are given—

(*a*) *Determination of uranium in ores*—Uranium has been determined in a variety of ores by the cellulose column method.[12] After removal of the ether, the aqueous remainder was heated with a few millilitres of perchloric acid and evaporated almost to dryness. The proposed method was then applied to the residue or an aliquot. The results are shown in Table I.

Table I

The determination of uranium in ores

Ore	Weight taken, g	Aliquot taken	U_3O_8 found, %	U_3O_8 present,[11] %
Monazite	0·198	10/50	0·381	0·37
	0·271	10/50	0·378	—
	0·223	10/50	0·360	—
Pyrochlore	0·561	—	0·0273	0·025
	0·477	—	0·0273	—
	0·549	—	0·0269	—
Siliceous ore	0·488	10/50	0·325	0·32
	0·264	10/50	0·358	—
	0·203	10/50	0·343	—
Columbite	0·471	—	0·0366	0·036
	0·653	—	0·0372	—
	0·565	—	0·0370	—
Monazite	0·205	—	0·114	0·12
	0·253	—	0·114	—
	0·287	—	0·113	—
	0·325	—	0·114	—
	0·229	—	0·111	—

(*b*) *Determination of uranium in thorium oxide*—Some preliminary experiments were made to see whether uranium could be quantitatively extracted from acidified thorium nitrate solutions into tributyl phosphate - carbon tetrachloride mixtures as uranyl nitrate. The tributyl phosphate - carbon tetrachloride mixture could then be removed, treated with EDTA and thiocyanate and the optical density measured. Some difficulties were found in this work. Nitric acid is extracted into the organic layer. This necessitates use of a buffering agent such as sodium acetate when the organic layer is removed and treated with thiocyanate. Also, 100 per cent. extraction was not obtained in one shaking. This meant that several portions of tributyl phosphate - carbon tetrachloride mixture would have to be used, mixed and diluted to a known volume. The sensitivity of the method would thereby be considerably decreased. The separation of the phases, too, was very poor. It was therefore decided to use a continuous ether extractor and determine the uranium in the extract.

REAGENTS—

Nitric acid, concentrated, 16 *N*.

Sodium fluoride solution—Dissolve 10 g of pure sodium fluoride in water and dilute to 500 ml.

Ammonium nitrate.

Saturated ammonium nitrate solution—Dissolve sufficient solid ammonium nitrate in warm water to form a saturated solution and cool to room temperature.

Perchloric acid, 12 *N*.

PROCEDURE—

Weigh 30 g of thorium oxide into a 400-ml beaker, and add 10 ml of sodium fluoride solution and 50 ml of concentrated nitric acid. Cover with a clock-glass and warm gently to boiling. Boil until the thorium oxide is dissolved, adding more nitric acid if necessary, and evaporate the solution until crystallisation commences. Cool somewhat, and add 10 ml of water and 5 g of ammonium nitrate. Cool the mixture to room temperature and dilute with water until all the solid is dissolved. Transfer the solution to the extraction vessel of a continuous ether-extraction apparatus, using saturated ammonium nitrate solution for washing out the beaker. Adjust the level of liquid in the extraction vessel to 2 cm below the side-arm. Cool the extraction vessel by means of an ice jacket.

Extract with a mixture of 50 ml of water and 100 ml of ether in the boiler flask for 1½ hours. Distil off the ether from the boiler flask and transfer the aqueous solution to a 250-ml beaker. Add 5 ml of concentrated nitric acid and 5 ml of perchloric acid and evaporate gently to fumes of perchloric acid. A small ashless filter-paper added to the evaporating solution often prevents bumping. Evaporate the solution nearly to dryness and transfer to a 100-ml beaker, using the minimum of water.

Determine the uranium present by applying the proposed method, but using 15 ml of 32·5 per cent. v/v tributyl phosphate in carbon tetrachloride and measuring the optical density in 4-cm cells. Determine the uranium present in parts per million.

RESULTS

A standard graph was prepared by extracting amounts of uranium equivalent to up to 200 μg of U_3O_8 into 15 ml of 32·5 per cent. tributyl phosphate - carbon tetrachloride mixture and measuring the optical density in 4-cm cells. An optical density of 1 was equivalent to 199·5 μg of U_3O_8. The standard deviation was 1·5 μg of U_3O_8.

A pure thorium oxide, with and without added uranium, was examined by this method. The results are shown in Table II.

TABLE II

DETERMINATION OF URANIUM IN THORIUM OXIDE

Weight of thorium oxide taken, g	Uranium oxide (U_3O_8) added, μg	Uranium oxide (U_3O_8) found, μg	Mean uranium oxide (U_3O_8), μg	Standard deviation, μg
30	nil	8·0, 5·4, 2·4, 7·2, 5·2, 2·8	5·1	2·3
30	40	44·7, 41·3, 43·3, 38·7	42·0	2·6

The result for no added uranium corresponds to a uranium content in the sample of 0·17 p.p.m. of U_3O_8, with a standard deviation of 0·08 p.p.m.

We are grateful to the Directors of Thorium Limited for permission to publish this paper and to Messrs. W. P. Kemp and K. W. Ponting for the preparation of the tributyl phosphate used in the work.

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The Determination of Thorium in Ores with APANS - *meso*Tartaric Acid Reagent after a Shortened Chromatographic Separation

By D. A. Everest and J. V. Martin

A rapid procedure is described for the determination of thorium in medium-grade and low-grade ores containing as much as 50 per cent. of zirconia. Thorium is separated from gross amounts of other elements by elution on a cellulose - alumina column and determined by a selective absorptiometric finish with APANS [1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic acid], *meso*tartaric acid being used as a masking agent for zirconium.

The determination of thorium in complex and low-grade ores by classical precipitation methods is extremely tedious and uncertain. In recent years attempts have been made to shorten the operational time involved by the introduction of selective solvent-extraction and chromatographic methods. Mesityl oxide[1,2,3] and tri-*n*-butyl phosphate have been the principal solvents used for the extraction of thorium, but such methods suffer from the disadvantage that thorium must be separated from zirconium by precipitation of thorium as oxalate or fluoride.[1,3]

The chromatographic procedure developed by Williams[4] has been used for the determination of thorium in a wide variety of minerals and ores. Thorium is separated from gross amounts of other elements by elution from a compound cellulose - alumina column with ether containing 12 per cent. v/v of nitric acid and is then precipitated as thorium oxalate and finally weighed as ThO_2. When the amount of thorium in the sample is low and the amount of zirconium is high, a double-column separation, *i.e.*, thorium is twice eluted on cellulose - alumina columns, is required. Alternatively, thorium has been separated from most of the zirconium present by a preliminary precipitation as fluoride and finally eluted from a cellulose - alumina column with ether - nitric acid solvent.[5] Small amounts of thorium thus separated have been determined by spectrographic[6] or colorimetric methods.[5,7]

The purpose of this work was to develop a method for the determination of thorium in which only one separation on a column would be required, and which should have an absorptiometric finish in which small amounts of impurities (particularly zirconium) present in the

eluate could be tolerated. Certain authors have criticised the original method of Williams on the grounds that recovery values for small amounts of thorium are low.[5,7] Guest[7] suggested that thorium is not quantitatively precipitated as hydroxide from aqueous solution after the first column separation, because of the presence of organic material extracted from the cellulose by the eluting solvent. Steele[5] claimed that thorium may be retained on the column by co-precipitation with titanium and zirconium in insoluble products of hydrolysis and in phosphates insoluble in nitric acid.

In view of the above-mentioned criticisms, the chromatographic procedure of Williams was examined and it was found that, for a single-column separation, no losses of thorium were encountered, even when the zirconium content of the sample was high. The method described in this paper is much more rapid than previous chromatographic procedures in that no lengthy chemical precipitations are needed and no double-column separation is required. The chromatographically separated thorium is determined absorptiometrically with APANS [1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic acid], *meso*tartaric acid being used as a masking agent for zirconium. APANS is a highly selective reagent for thorium and is now widely used.[2,3,8,9,10] Zirconium is one of the few metals that interferes appreciably, and Grimaldi and Fletcher have recently found that its interference may be reduced by using D(+)-tartaric acid as a masking agent.[11] These authors also made brief mention of *meso*-tartaric acid, which has been found in this work to mask satisfactorily any zirconium eluted from a cellulose - alumina column. Since the disodium salt of ethylenediaminetetra-acetic acid (EDTA) is known to form a very stable complex with zirconium, it also was examined as a possible masking agent, but was found to be unsatisfactory.

The procedure was developed principally because of a need to determine thorium in experiments on the amenability of certain thorite-bearing tailings, containing approximately 5 per cent. of ThO_2, to treatment with mineral acid. The tailings contain 50 to 70 per cent. of zircon and the residue for analysis contains almost all the zircon and only small amounts (less than 0·5 per cent.) of thorium. Because, however, the accuracy attainable with APANS ($\pm$0·5 to 1·0 per cent.) is comparable with that by other methods at present used for thorium, application has also been made to the determination of thorium in materials containing as much as 10 per cent. of ThO_2.

Titration of thorium with EDTA[12] after elution from a cellulose - alumina column has also been examined as a rapid method for the determination of the metal in medium-grade ores. The results have been good for some ore samples, but the titration is liable to interference by traces of impurities, *e.g.*, zirconium and phosphate, eluted from the column. Because of its limited application, the titration method is inferior to the APANS - *meso*-tartaric acid procedure.

Experimental

Chromatographic separation—

The method used for the breakdown of the ore and for the chromatographic elution is that described by Williams,[4] starting at p. 301, line 16, and finishing at p. 302, line 17, of his paper. Certain operations in the method were found to be of particular importance for samples of high zirconium content. The sodium phosphate added to the solution containing the thorium should be thoroughly stirred into the warmed solution, otherwise much will remain undissolved in the gelatinous mass formed by precipitated zirconium phosphate. Sufficient alumina should be added to this solution to give a dry wad, so that the gelatinous mass of zirconium phosphate is broken up and well distributed in the alumina.

Recovery of macro amounts of thorium from excess of zirconium by the column method—

Because of the criticism by certain authors of the cellulose - alumina column procedure for thorium, the recovery of thorium in the presence of excess of zirconium was first checked for macro amounts of thorium (25 mg of ThO_2). After a single-column chromatographic separation, thorium was precipitated as oxalate, ignited and weighed as ThO_2. The recovery of thorium from a mixture with zirconium (containing 350 mg of ZrO_2) was 99 per cent., which suggested that, for the single-column procedure at least, there are no serious losses of thorium.

The recovery of smaller amounts of thorium is discussed subsequently.

COLORIMETRIC DETERMINATION OF THORIUM WITH APANS WITH USE OF A MASKING AGENT FOR ZIRCONIUM—

As has already been mentioned, the masking effect of EDTA and of *meso*tartaric acid on the characteristic interference of zirconium with the absorptiometric procedure with APANS for thorium was studied. The results are shown in Table I. To the stated amount of thorium were added by pipette the masking agent, 1 ml of concentrated hydrochloric acid, 5 ml of 0·1 per cent. w/v aqueous APANS solution and 20 ml of absolute ethanol, in that order, and the solution was made up to 50 ml. The blank solution contained all the reagents except the masking agent.

TABLE I

REAGENTS FOR MASKING ZIRCONIUM IN THE ABSORPTIOMETRIC DETERMINATION OF THORIUM WITH APANS

Masking agent	Composition of mixture: ThO_2, mg	Composition of mixture: ZrO_2, mg	Optical density at 550 mμ
No masking agent	0·302		0·417
	0·302	0·2	0·538
0·2 ml of 0·025 *M* EDTA solution	0·302		0·416
	0·302	0·2	0·375
1 ml of 5 per cent. w/v *meso*tartaric acid solution	0·302		0·418
	0·302	0·2	0·409
	0·302	1	0·409
	0·302	2	0·414

With no masking agent present zirconium causes a high positive interference. In the system containing EDTA, zirconium induces a small negative interference similar to that observed by Grimaldi and Fletcher[11] with D(+)-tartaric acid. With *meso*tartaric acid present as masking agent, a similar negative interference, which is however negligibly small, is observed, and as much as 2 mg of ZrO_2 can be successfully masked. When larger amounts of zirconium are present, the red precipitate of the zirconium - APANS complex is slowly formed and optical-density measurements are impossible. This precipitation is not prevented by increasing the concentration of *meso*tartraric acid by a factor of 2·5.

*meso*Tartaric acid, however, appeared to be a very satisfactory reagent for masking zirconium and was therefore used in the determination of thorium with APANS in subsequent work. The relatively slight interference of ferric iron in this determination is not eliminated with *meso*tartaric acid, and hydroxylamine hydrochloride was used to reduce iron to the non-interfering ferrous state.

ABSORPTIOMETRIC DETERMINATION OF THORIUM WITH APANS - *meso*TARTARIC ACID REAGENT AFTER CHROMATOGRAPHIC SEPARATION—

The absorptiometric method with APANS and with *meso*tartaric acid as masking agent for zirconium was applied to the determination of thorium separated by the cellulose - alumina column procedure. A high degree of accuracy was achieved for the determination of as little as 0·2 mg of ThO_2 in as much as 500 mg of ZrO_2. Thorium was also determined satisfactorily in a number of low-grade and medium-grade ores.

METHOD

REAGENTS—

Hydroxylamine hydrochloride solution—A 10 per cent. w/v aqueous solution.

meso*Tartaric acid solution*—A 5 per cent. aqueous solution of the monohydrate.

APANS solution—A 0·1 per cent. w/v aqueous solution of the sodium salt of 1-(*o*-arsonophenylazo)-2-naphthol-3:6-disulphonic acid.

PROCEDURE—

After the chromatographic separation of thorium, add 150 ml of water and several glass beads to the nitric acid - ether eluate, and evaporate the ether by warming on a steam-bath. Boil the remaining aqueous solution to small bulk, transfer it to a calibrated flask and make up to the mark with 25 per cent. v/v nitric acid (see Note 1). By pipette, put a suitable aliquot (see Note 2) into a 100-ml beaker and evaporate to dryness. Add 2 ml of a mixture of 70 per

cent. perchloric acid, concentrated nitric acid and water (2 + 1 + 1) and heat to dryness. Add by pipette 1 ml of concentrated hydrochloric acid, cover the beaker with a watch-glass, and warm gently to dissolve the residue. Dilute to about 10 ml, add 1 drop of hydroxylamine hydrochloride solution and heat to a point just short of boiling to reduce ferric iron. Cool the solution and transfer it to a 50-ml calibrated flask. Add by pipette 1 ml of *meso*tartaric acid solution, 5 ml of APANS solution and 20 ml of absolute ethanol in that order; allow to cool and make up to the mark. Measure the optical density in 1-cm glass cells at 550 mμ with a Unicam spectrophotometer (red cell) against a blank solution containing all reagents. (See Note 3.)

Beer's law is obeyed between 0 and 0·5 mg of ThO_2. It is advisable to prepare a separate calibration graph for each batch of stock APANS solution made.

NOTES—

1. Thorium may form an insoluble precipitate with organic matter resulting from degradation of the cellulose if the details of this step are not carefully followed.
2. Not more than 0·4 of the total volume should be taken. With larger aliquots sufficient zirconium may be present to cause immediate precipitation with APANS.
3. Optical-density readings are made 1 hour after the preparation of the thorium - APANS solutions except for solutions from low-grade samples, containing much zirconium. For these, optical-density readings are made after 15 to 20 minutes, since precipitation of the zirconium - APANS complex may occur if the solutions are set aside for a longer period.

RESULTS

DETERMINATION OF SMALL AMOUNTS OF THORIUM IN ARTIFICIAL MIXTURES WITH ZIRCONIUM—

Solutions containing thorium and zirconium were prepared from solutions of the metal nitrates. The mixed solutions were evaporated just to dryness, the residue was dissolved in 20 ml of 25 per cent. v/v nitric acid and the normal single-column procedure was followed. Thorium in the eluate was determined as described above. The results for thorium in the presence of 500 mg of ZrO_2 were as follows—

Thorium present (ThO_2), mg ..	1·01	0·97	0·97	0·20	0·20
Thorium found (ThO_2), mg ..	1·01	0·98	0·98	0·22	0·22

PURIFICATION OF ZIRCONIUM—

In preliminary experiments zirconium was not purified and the recovery values were high in the determination of small amounts of thorium. This apparent error was subsequently found to be due to the presence in the zirconium of traces of thorium as an impurity. The zirconium was therefore purified by the following method, which is based on the procedure used by Steele[5] for the determination of thorium in zirconium—

Twenty-five grams of zirconium nitrate were warmed with 50 ml of concentrated hydrofluoric acid in a polythene beaker for 1 hour. Then 100 ml of water were added and the solution was warmed on a steam-bath for 2 hours to complete the dissolution of the zirconium. The solution was diluted to 200 ml with water and cooled. Next, 4 ml of lanthanum nitrate solution (containing 10 mg of La_2O_3 per ml), 80 ml of mercurous nitrate solution (a 0·95 per cent. w/v $Hg_2(NO_3)_2$ in 0·1 per cent. v/v nitric acid) and 8 ml of 7 per cent. v/v hydrochloric acid were added in that order. The solution was set aside overnight, a small amount of cellulose pulp was added and the mixture was filtered through a Whatman No. 540 filter-paper in a polythene funnel into a large platinum dish. The filtrate was evaporated to dryness and the residue was heated to fumes several times with sulphuric acid. The residue was dissolved in water and the solution was diluted to 500 ml; zirconium was precipitated as the hydroxide with ammonia solution. The precipitate was collected in a Buchner funnel and dissolved in nitric acid, and zirconium hydroxide was re-precipitated five times to remove sulphate ions. The hydroxide was dissolved in 100 ml of concentrated nitric acid and the solution was diluted to 500 ml and filtered through Whatman No. 42 filter-paper. To the filtrate 275 ml of concentrated nitric acid were added, and the solution was cooled and made up to 1 litre with water. The zirconium content was found gravimetrically by evaporating an aliquot of the solution and igniting to the oxide, ZrO_2.

DETERMINATION OF THORIUM IN LOW-GRADE AND MEDIUM-GRADE ORES—

Thorium was determined in a number of residues from the mineral-acid leaching (with nitric and sulphuric acids) of thorite-bearing tailings. The zircon content was between 50

and 70 per cent. in all the residues. Each analysis was performed in duplicate on 1-g samples and the results were confirmed by spiking duplicate samples with a known amount of thorium, approximately equal to that already present in the ore. The ore was spiked after the extraction of the potassium hydroxide melt with nitric acid by the addition of a known volume of standard thorium nitrate solution. The results were as follows—

Residue No.	1	2	3	4	5
Thorium (ThO_2) found by direct determination, %	0·26, 0·26	0·35, 0·36	0·44, 0·45	0·30, 0·30	0·22, 0·22
Thorium (ThO_2) found by determination after spiking, %	0·26, 0·26	0·36, 0·35	0·41, 0·45	0·29, 0·28	0·21, 0·21

Thorium was also determined in medium-grade ores and the results are compared in Table II with those obtained by the cellulose - alumina column procedure with an oxalate finish.[4]

TABLE II

DETERMINATION OF THORIUM IN MEDIUM-GRADE ORES

Sample	Thorium (ThO_2) found by APANS - *meso*tartaric acid finish,* %	Thorium (ThO_2) found by oxalate finish,* %
Indian monazite	9·85	9·85
African monazite	6·40	6·40
Thorite	6·55	6·55
Monazite	7·60	7·45
African monazite concentrate	15·50	15·50
Thorium containing gold residue	12·50	12·20
Asian monazite	6·95	7·00

* Mean values are given for a number of determinations.

APPENDIX

Since the completion of this work, Grimaldi, Jenkins and Fletcher have published papers[13,14] about the determination of thorium in silicate rocks and in ores by using a system containing APANS and *meso*tartaric acid after preliminary precipitation of thorium as iodate.

We thank Mr. G. H. Smith and other members of the Analytical Survey Group for supplying certain of the analytical results.

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The Use of Selective Desorption from Carbon Columns for the Determination of Dextrose and Maltose in Starch Conversion Products

By STELLA J. PATTERSON and R. I. SAVAGE

A method is proposed for determining the mono- and disaccharides in starch conversion products, based on selective desorption followed by elution from a carbon column. Recovery is quantitative provided the most suitable temperature conditions for recovery from each batch of carbon are established before it is put into routine use. The method of testing the carbon in order to obtain these conditions is described.

The classical methods available for determining individual sugars in a mixture such as occurs in starch conversion products depend on reducing power towards cupric salts under various conditions and on optical rotation. In these methods inaccuracies arise because of the effect of the other sugars present on the sugar being determined, and it is not easy to make allowance for this effect. A method that physically separates the sugars one from another is therefore desirable.

Selective desorption from a carbon column provides such a method, and has been used by a number of workers. A mixture of sugars is adsorbed on a column of carbon, and the individual sugars are then removed selectively by washing successively with water and with aqueous ethanol of increasing concentrations. In this way, monosaccharides are separated first, then disaccharides, trisaccharides and so on. Most of the methods published, however, are inconvenient for routine quantitative use, many of them depending on gradient elution of the sugars from the column or on forcing the liquid through the column by pressure from a gas cylinder, and in much of the work done the American carbon Darco G 60 has been used. Further, some of the procedures described did not yield quantitative recovery of the sugars.[1,2,3]

In this paper a test for the suitability of a carbon for use in the columns is described. Of the carbons we have investigated, so far only one, B.D.H. (British Drug Houses Ltd.) "Activated Charcoal, Acid Washed," has been found to give quantitative recovery of sugars when used as supplied. The capacity of this carbon for retaining sugars is such that, in order to be certain of recovering the separated sugars quantitatively in sharp bands uncontaminated by other sugars, the concentration of sugar in the eluates has to be too low for accurate determination by the usual macro methods, although the semi-micro method of Somogyi[4,5] is suitable. We found it of advantage to modify Somogyi's improved reagent by reducing the recommended amount of sodium sulphate in order to avoid the crystallisation that took place at the temperatures normally prevailing in this country. This modified reagent has been satisfactory for determining sugars in the eluates, and we have had no evidence that its keeping qualities have been impaired. During very cold weather, crystallisation of sodium sulphate occasionally occurs overnight with this modified reagent, which is saturated with sodium sulphate at approximately 15° C. Warming the solution to redissolve the crystals has not affected its reduction equivalent, and we have thought it undesirable to reduce any further the amount of sodium sulphate present. For quantitative work, we have found this carbon-column method more convenient than elution of the sugars from paper chromatograms. The immediate object of our work has been to provide a method for determining the amounts of mono- and disaccharides in liquid glucose and similar starch conversion products; by making use of the same principles, it should be possible to extend the method to include the determination of higher saccharides and other mixtures of sugars.

It was decided that it would be of advantage if we could use a "blind" method, that is, one in which we knew that the dextrose was all being eluted into one fraction and the maltose all into another, instead of collecting a number of fractions to make sure of obtaining all of one sugar and none of another.

The first batch of B.D.H. charcoal that we tried worked satisfactorily under the conditions described at laboratory temperature, but we had difficulty with subsequent batches. As shown later in the paper, this can be overcome by eluting at a suitable temperature.

METHOD

APPARATUS—

The assembly of the carbon column is shown in Fig. 1.

A 6-inch × ¾-inch test-tube has a hole blown in its end, and to this is fused a short length of 4-mm glass tubing. This forms the adsorption column.

The rubber bung, A, fits either a standard 100 to 110-ml sugar or Reichert - Polenske flask or a 6-inch × ¾-inch test-tube, which can be changed by slowly opening tap B to the air and rapidly substituting the replacement.

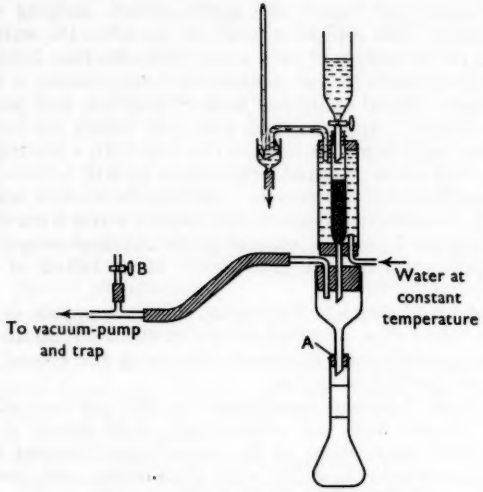

Fig. 1. Assembly of carbon column

Some device for maintaining the column at the required temperature is necessary. We use a Liebig condenser type of jacket surrounding the column (as shown in Fig. 1), through which water is circulated from a coil immersed in a beaker of water and heated or cooled as required.

For the Somogyi determinations, a series of glass-stoppered boiling-tubes, or ordinary boiling-tubes with fitted corks, is required. To hold the boiling-tubes rigidly in a boiling-water bath, we use a rack of Terry's clips that can be secured on either side of the bath.

REAGENTS—

Activated carbon—The product supplied by the British Drug Houses Ltd., "Activated Charcoal Powder for Decolorising Purposes. Washed with Acid."

Kieselguhr—We used Metasil A. Other brands have been found to be equally suitable.

Aqueous ethanol, approximately 7 per cent. v/v—Dilute 75 ml of aldehyde-free 95 per cent. ethanol to 1 litre with water.

Modified Somogyi micro-copper reagent—Dissolve 30 g of potassium sodium tartrate and 30 g of anhydrous sodium carbonate in about 200 ml of hot water and add 40 ml of *N* sodium hydroxide solution and, with stirring, 80 ml of a 10 per cent. w/v solution of copper sulphate, $CuSO_4.5H_2O$. Boil the solution to expel air. Dissolve 290 g of sodium sulphate, $Na_2SO_4.10H_2O$, in about 300 ml of water and boil the solution to expel air; then add it to the copper solution contained in a 1-litre calibrated flask. Add 8 g of potassium iodide dissolved in a few millilitres of water, and then 10·0 ml of *N* potassium iodate. Dilute to the mark with air-free water. If the solution is not perfectly clear, filter it.

Sulphuric acid, approximately 6 N—Dilute 170 ml of concentrated sulphuric acid to 1 litre with water.

Sodium thiosulphate solution, approximately 0·005 N.

PROCEDURE FOR ROUTINE DETERMINATIONS—

Place a small plug of cotton-wool inside the column, press it well down, and moisten it thoroughly with water. Make a slurry of about 0·1 g of kieselguhr with water, pour it into the tube, and allow it to drain.

Make a slurry of 7 g of a mixture of equal parts by weight of carbon and kieselguhr with water, taking care that every particle is wetted, and pour it into the tube. Apply gentle suction. (From this time onwards the top of the carbon column must never be allowed to become dry.) When only a 1-mm layer of supernatant liquid remains, apply a circle of filter-paper to the top of the column and press down gently. Pour 100 ml of water at the required temperature into the separating funnel, and apply suction, keeping about a 1-cm head of liquid on top of the column. The column is ready for use when the water has passed through.

Prepare a solution of the sample of such a concentration that 5 ml will contain approximately 10 mg of anhydrous maltose; for commercial liquid glucose a 2 per cent. solution is suitable. Place an empty 100-ml calibrated flask in position, and put 5 ml of the solution by pipette on to the column. Apply suction and, just before the last of the solution disappears into the column, wash down the walls of the tube with a few millilitres of water; then elute the column with water at the required temperature until 95 to 100 ml of eluate containing the dextrose have been collected in the receiver. Release the suction, and substitute an empty 100-ml calibrated flask as receiver. Remove the surplus water from the top of the column with a pipette, and substitute 7 per cent. ethanol at the required temperature for water in the separating funnel. Apply suction again, and collect 95 to 100 ml of ethanolic eluate containing the maltose.

The rate of flow of liquid through the column does not appear to influence the elution of the sugars; we have found that a rate of 100 ml in 40 to 50 minutes is convenient.

Dilute the aqueous and ethanolic eluates to volume in the 100-ml flasks and determine the sugars by Somogyi's method as follows.

By pipette put duplicate 5-ml portions of (*a*) water, (*b*) 7 per cent. ethanol, (*c*) the aqueous and (*d*) the ethanolic eluates from the column into eight 6-inch × 1-inch boiling-tubes. Then also by pipette put 5-ml portions of the micro-copper reagent into each tube. Mix thoroughly, cover the mouths of the tubes with glass bulbs, and immerse the tubes in a bath of briskly boiling water. Withdraw the four tubes containing the aqueous eluates after exactly 10 minutes and the four containing the ethanolic eluates after a further 10 minutes, and cool them to room temperature in a bath of cold water. To each tube add approximately 1 ml of 6 *N* sulphuric acid, drawing the glass bulb aside just sufficiently to admit the jet of the pipette. The acid should be rapidly squirted, rather than permitted to flow into the test-tube, so that the entire contents of the tube are mixed and acidified at once. A teat pipette is convenient for this purpose. Immediately swirl the tube vigorously until no trace of cuprous oxide remains undissolved. Set the tube aside for 2 minutes, swirl again, wash down the glass bulb with water into the tube, mix well, and titrate slowly with 0·005 *N* sodium thiosulphate, mixing well during the early stages of the titration after each addition of 0·5 ml of sodium thiosulphate. Add freshly prepared starch indicator near the end-point, and shake thoroughly just before the final drop is added. Read the burette to 0·01 ml (we find a 25-ml grade A burette suitable). Duplicates should agree within 0·02 ml. (In theory it would be no doubt more correct to carry out blank determinations with water and ethanol that had passed through the column, but in practice we have found that results determined in this way are the same as those given when the water and ethanol are used direct.)

Standardise the sodium thiosulphate against 0·005 *N* potassium iodate.

Calculate the differences between the titres of the sugar solutions and the corresponding blanks in terms of 0·005 *N* sodium thiosulphate, and read off the corresponding weights of sugar from the relevant graphs.

It is preferable for each worker to construct his own graph showing the relation between volume of 0·005 *N* sodium thiosulphate in ml and amount of sugar in mg. The results shown below were obtained from several closely agreeing determinations made in this laboratory—

Anhydrous dextrose (10 minutes' heating) mg ..	1·33	1·0	0·667	0·5	0·4	0·2
Volume of 0·005 *N* sodium thiosulphate, ml ..	8·75	6·47	4·27	3·12	2·45	1·15
Anhydrous maltose (20 minutes' heating), mg ..	2·0	1·5	1·0	0·667	0·4	0·2
Volume of 0·005 *N* sodium thiosulphate, ml ..	7·51	5·58	3·63	2·36	1·365	0·635

The purity of the sugars used in constructing the graphs was tested by paper chromatography and polarimetry, and the appropriate corrections were made for moisture.

Testing of the method

Mixtures prepared from anhydrous dextrose and maltose were quantitatively separated by the proposed method; for a mixture containing 10·0 mg of each sugar, 10·1 mg of each were found, and similarly for a mixture containing 14·0 mg of each sugar, 14·2 mg of each were found. Further, analyses of samples of liquid glucose gave reproducible figures that were in agreement with the results obtained by quantitative paper chromatography.

In another experiment the aqueous and the ethanolic eluates from the column were collected in successive 5-ml portions, each of which was separately analysed for sugar. The results are illustrated in Fig. 2.

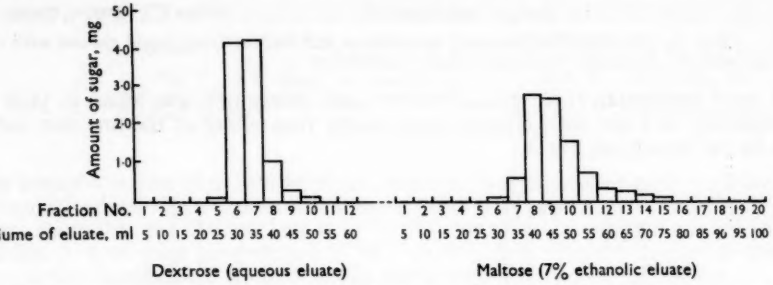

Fig. 2. Histogram for the recovery of dextrose and maltose from liquid glucose with use of batch No. 1 B.D.H. charcoal at room temperature

It will be seen that all the dextrose was contained in the first 50 ml of water and all the maltose in the first 75 ml of 7 per cent. ethanol. The 100 ml of each prescribed in the method therefore allowed adequate "clearance." Moreover, there is no indication that the later ethanolic fractions were eluting any maltotriose.

After one of the routine determinations of dextrose and maltose in liquid glucose, 100 ml of 95 per cent. ethanol were passed through the column and collected separately. The remaining 90 ml of the aqueous and the 7 per cent. ethanolic eluates, and the 95 per cent. ethanol, were separately evaporated to dryness, and the residues were taken up in 1 drop of water and submitted to paper chromatography. The aqueous portion was found to contain dextrose with possibly a minute trace (less than 0·005 mg) of maltose, the 7 per cent. ethanolic portion contained only maltose and the concentrated ethanol portion, which contained triose, tetrose and so on, gave no trace of either dextrose or maltose. (It was necessary to evaporate the maltose portion in a partial vacuum at 70° C, since evaporation to dryness at 100° C caused transformation of the maltose into a complex mixture of sugars.)

We recommend that the amount of maltose put on to the column should be about 10 mg. It is possible to put on considerably more than this amount before the column is so overloaded that some of the maltose appears in the 100-ml aqueous eluate with the dextrose, but continued washing with large volumes of water after all the dextrose has been eluted will slowly remove maltose from a heavily over-loaded column. By restricting the amount of maltose put on to the column to about 10 mg, we have ensured that none of it is eluted by the first 200 ml of water, *i.e.*, by 100 ml in excess of the amount of water specified for eluting the dextrose.

Most commercial liquid glucose contains a small amount of sulphur dioxide. A liquid glucose was specially prepared without the addition of sulphur dioxide, a considerable excess of sulphur dioxide over the amount normally present was added, and the dextrose and maltose were determined at intervals during several months. The presence of the sulphur dioxide had no effect on the accuracy of the method.

Variations between different batches of carbon

It was found on using a second batch of B.D.H. charcoal of the same description as the first, that, although the sugars could be recovered quantitatively as before, the recommended 95 to 100 ml of 7 per cent. ethanol were barely sufficient for complete elution of the whole of the maltose. The histogram shown in Fig. 3, which was obtained by collecting successive 10-ml fractions of aqueous and ethanolic eluates containing dextrose and maltose, illustrates this.

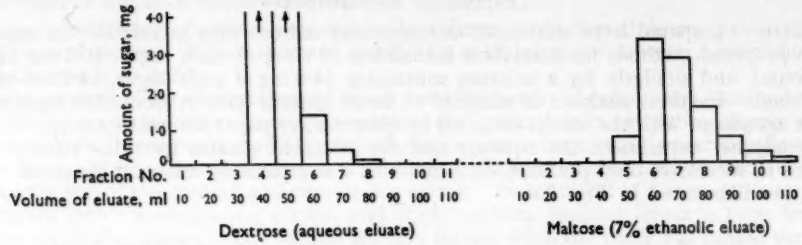

Fig. 3. Histogram for recovery of dextrose and maltose from liquid glucose with use of batch No. 2 B.D.H. charcoal at room temperature

A third batch of B.D.H. charcoal of the same description was found to yield maltose quantitatively to 7 per cent. ethanol more readily than either of the first two batches, as shown by the histogram, Fig. 4.

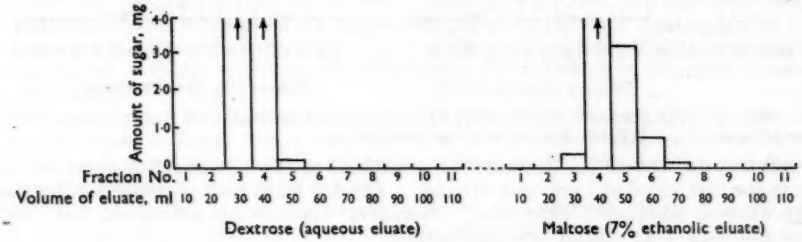

Fig. 4. Histogram for recovery of dextrose and maltose from liquid glucose with use of batch No. 3 B.D.H. charcoal at room temperature

In view of this variability from batch to batch of carbon, it appears advisable to test each batch by collecting the eluates in fractions initially before putting it into routine use, to determine the conditions most suitable for ensuring a clean separation of sugars in convenient volumes of eluate. An initial check of this type also has the advantage of providing a ready test for types of carbon other than the one we recommend, and the same principle could be extended to the elution of higher sugars with other concentrations of aqueous ethanol.

We found that the volume of 7 per cent. ethanol required for complete removal of maltose from a column is considerably influenced by temperature, as the histograms in Figs. 5, 6, 7 and 8 show.

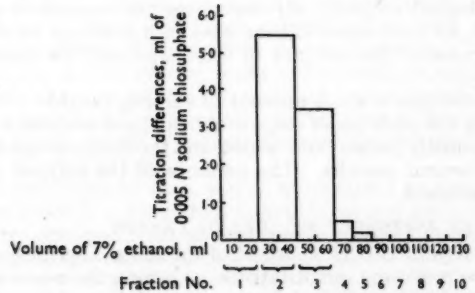

Fig. 5. Histogram for recovery of maltose from batch No. 2 B.D.H. charcoal at 40° C

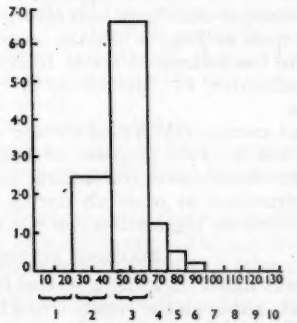

Fig. 6. Histogram for recovery of maltose from batch No. 2 B.D.H. charcoal at 30° C

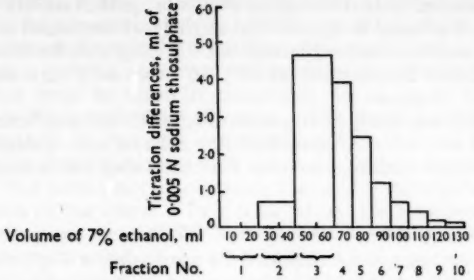

Fig. 7. Histogram for recovery of maltose from batch No. 2 B.D.H. charcoal at 20° C

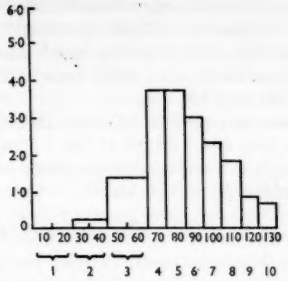

Fig. 8. Histogram for recovery of maltose from batch No. 2 B.D.H. charcoal at 10° C

It is largely a matter of individual choice whether the temperature or the volume of the eluting liquid is adjusted in order to obtain satisfactory results from any one carbon. These histograms show that if a 100-ml flask is to be used for collecting maltose washed out from charcoal No. 2, a working temperature of 30° C is only just sufficient to give quantitative recovery; if the temperature is significantly below this, the results will be low.

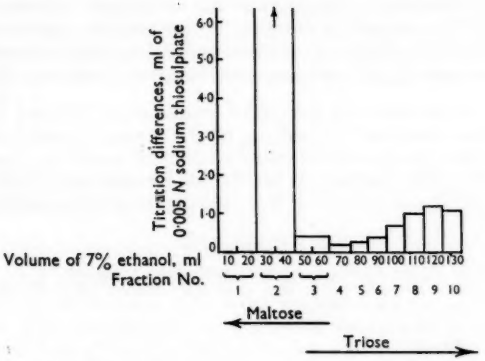

Fig. 9. Histogram for recovery of maltose from batch No. 3 B.D.H. charcoal at 30° C

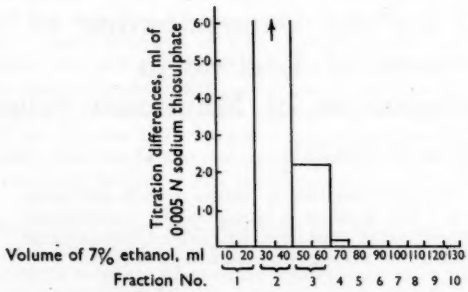

Fig. 10. Histogram for recovery of maltose from batch No. 3 B.D.H. charcoal at 16° C

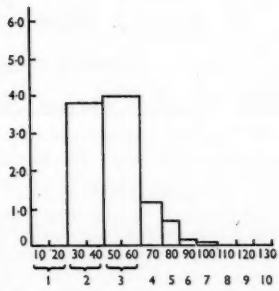

Fig. 11. Histogram for recovery of maltose from batch No. 3 B.D.H. charcoal at 10° C

We have not experienced any difficulty in recovering dextrose quantitatively from a carbon column in 100 ml of water. Recovery is speeded up slightly at increased temperatures, but the effect is very much less marked than with maltose. Histograms for the elution of maltose from the third batch of B.D.H. charcoal at 30°, 16° and 10° C are shown in Figs. 9, 10 and 11.

When this carbon from batch No. 3 was used, all the maltose appears to have been eluted into the first 40 to 60 ml at 30° C, and was followed immediately by the triose. Clearly, 30° C is too high a working temperature for this carbon, the most suitable being the normal room temperature of 15° to 18° C.

Procedure for testing a batch of carbon

The histograms at various temperatures were obtained by preparing a 7-g column as usual with equal parts of the carbon under test and kieselguhr, and washing with 100 ml of water. By pipette, 5 ml of a 2 per cent. solution of liquid glucose were put on to the column, and the dextrose was removed, either into 100 ml of water or into the required fractions, numbered 6-inch × ¾-inch test-tubes graduated at 10 ml and 20 ml being used. Then surplus water was removed from the top of the column, 7 per cent. ethanol was substituted for water in the tap-funnel, and the ethanol fractions were collected. All solutions were heated or cooled to the working temperature before being passed through the column.

The usual Somogyi sugar determinations were carried out on 5 ml of each fraction, and the ordinates of the histograms are differences between titration and blank values. Differences in titration of less than 0·1 ml of 0·005 *N* sodium thiosulphate have been disregarded.

Quantitative recovery cannot be expected when the eluate is split up into many fractions; the histograms merely show the position at which the sugars are recovered. When a new carbon is used, it is also necessary to confirm, by putting through known amounts of pure sugars, collecting 100 ml each of aqueous and ethanolic eluates and carrying out a quantitative determination at the selected temperature, that recovery is in fact quantitative.

We are grateful to members of the Sub-Committee of Subject 26 (Starch-Conversion Products) of the British National Committee of the International Commission for Uniform Methods of Sugar Analysis for the considerable amount of work they have done in testing the proposed method, to Mr. J. L. Buchan, M.Sc., for his interest and advice, and to the Government Chemist, Dr. G. M. Bennett, C.B., F.R.S., for permission to publish this paper.

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A Rapid Method for the Determination of Small Amounts of Lactose in Milk and Tissue Suspensions of Mammary Gland

By T. F. SLATER

A method is described for the determination of lactose in milk and tissue suspensions of mammary gland. The method is based on the development of a green colour on heating lactose, orcinol and ferric ions in concentrated acid solution. The method is applicable to microgram amounts of lactose.

Lactose is the only carbohydrate occurring in normal cow and rat milk in other than trace concentrations.[1,2,3,4] Lactose can, therefore, be determined in them by a non-specific method of carbohydrate determination. The normal routine methods used for the determination of lactose in milk, for instance, reduction of cupric salts in alkaline solution and

the chloramine-T and polarimetric methods, are not suitable for the determination of extremely small concentrations of lactose. Further, the methods are time-consuming. Colorimetric methods described for the determination of lactose and applicable to the rapid analysis of numerous samples are either not simple enough for routine practice or have involved critical stages that must be carefully controlled, for example, the ferric chloride - sodium carbonate method of Mitra and Roy,[5] the tetrazolium method of Mattson and Jensen[6] and the methylamine method of Malpress and Morrison.[7]

Similar difficulties arise with determinations of lactose on tissue suspensions of mammary gland. Rat mammary gland tissue contains a variable content of milk retained in the ducts and alveoli of the gland. In full lactation, the retained milk may account for up to 60 per cent. of the gross wet weight of the gland.[8] This retained milk contributes appreciably to the total nitrogen content of the whole tissue homogenate, hence invalidating the direct usage of total nitrogen values as a measure of the tissue present in the sample.[9] It is therefore of importance, in quantitative experiments involving the mammary gland, to correct analyses on rat mammary tissue for the milk retained in excised gland. Again, normal methods are not suitable for determinations of lactose in small samples of rat mammary tissue suspensions in which the amount of lactose present may be less than 100 μg per sample.

Orcinol (3:5-dihydroxytoluene) reacts with pentoses in the presence of ferric ions and concentrated hydrochloric acid to give an intensely green chromogen.[10] This is the basis of the standard method for estimating ribonucleic acid in animal tissues.[11,12] Orcinol also reacts with other carbohydrates under the conditions outlined. Hexoses[13] and disaccharides, for instance, give brownish hazy solutions and, as the concentration of the sugar is increased, a heavy brown deposit forms, which is soluble in ethanol to give a clear yellow-brown solution. The orcinol reaction has been adapted in this investigation to provide a quantitative method for determining lactose in the absence of other carbohydrates. The colorimetric method described below enables 5 to 200 μg of lactose to be determined rapidly and accurately in milk and mammary gland suspensions; further, a number of determinations can readily be performed concurrently.

Experimental

An investigation of the variables in the lactose - orcinol reaction was carried out in order to find the optimal conditions for the reaction.

Variables in the lactose - orcinol reaction—

Absorption curve—One millilitre of a lactose solution (500 μg per ml in water) was heated on a boiling-water bath for 50 minutes with a 0·1 per cent. solution of ferric chloride in concentrated hydrochloric acid containing 0·5 per cent. of orcinol. After the solution had cooled, ethanol was added to clear the haze and the absorption spectrum was determined by means of a Beckmann SP500 spectrophotometer, 1-cm cells being used. The absorption spectrum is shown in Fig. 1 (*a*).

Stability of the colour—The stability of the colour formed is illustrated by the fact that the extinction at 670 mμ decreased by approximately 4 per cent. in 24 hours and the extinction at 440 mμ increased by approximately 6 per cent. in 24 hours.

Boiling time—Mixtures of 3 ml of a 0·1 per cent. solution of ferric chloride in concentrated hydrochloric acid, 0·3 ml of a 10 per cent. solution of orcinol in ethanol and 1 ml of a solution of lactose (approximately 300 μg per ml in water) were boiled for different times in a water bath. Fig. 1 (*b*) shows how the absorptions at 440 mμ and 670 mμ increased with boiling time. It can be seen that the green chromogen (wavelength maximum 670 mμ) was formed more rapidly than the brown chromogen (wavelength maximum 440 mμ) and reached a plateau after 10 minutes' boiling. The positions of the absorption maxima did not vary with boiling time.

Concentration of orcinol—Mixtures of 3 ml of a 0·1 per cent. solution of ferric chloride in concentrated hydrochloric acid, 1 ml of a solution of lactose (200 μg per ml in water), x ml of orcinol solution of various concentrations in 95 per cent. ethanol and $(1 - x)$ ml of water were boiled for 15 minutes in a water bath. After the solutions had cooled, the absorptions at 670 mμ were measured. The extinctions of the solutions increased as the concentration of orcinol increased and reached a plateau for final orcinol concentrations over 1 per cent.

Concentration of ferric ions—The influence of ferric ions on the lactose - orcinol reaction is shown in Fig. 1 (*c*). Ferric chloride solutions of various concentrations in concentrated hydrochloric acid were prepared and 3 ml of each solution were boiled for 15 minutes with 0·5 ml of a 10 per cent. solution of orcinol and 1 ml of a solution of lactose (200 μg per ml in water). After the solution had cooled, 2 ml of ethanol were added and the absorption at 670 mμ was measured.

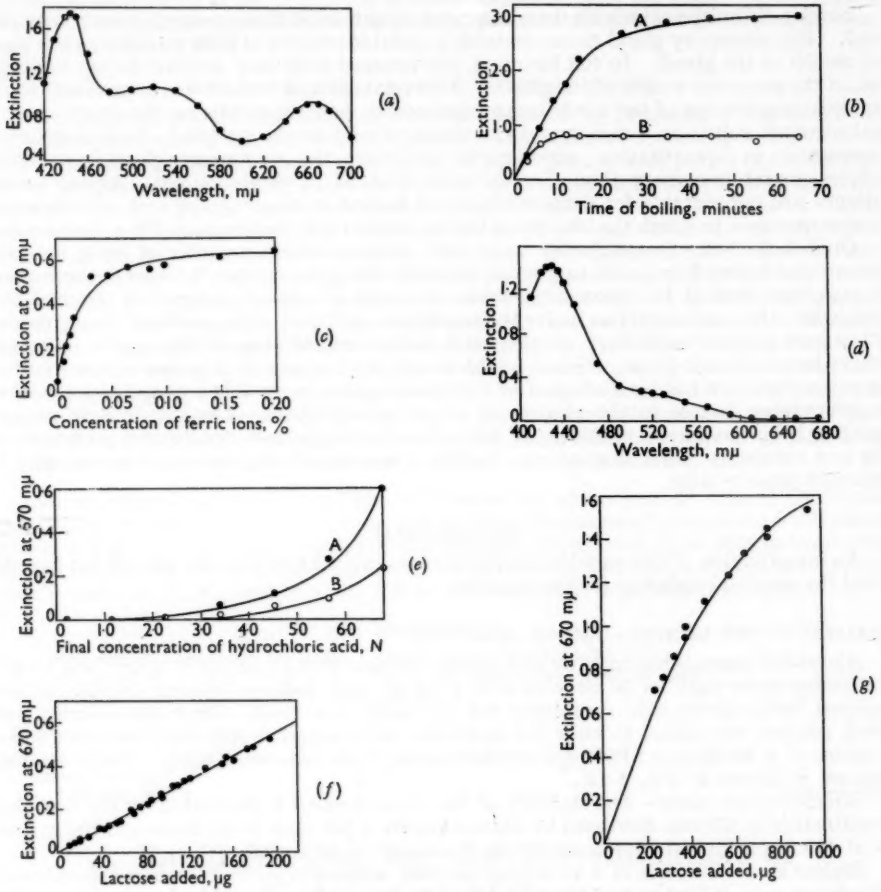

Fig. 1. Variables in the lactose - orcinol reaction

Fig. 1(*a*). Absorption spectrum of the solution obtained by heating lactose, orcinol, ferric chloride and concentrated hydrochloric acid

Fig. 1(*b*). Effect of boiling time on the extinction: curve A, at 440 mμ; curve B, at 670 mμ

Fig. 1(*c*). Effect of the concentration of ferric ions on the extinction at 670 mμ. The concentration of ferric ions refers to the concentration of ferric chloride in the 3 ml of ferric chloride - hydrochloric acid solution added

Fig. 1(*d*). Effect of 2:2′-dipyridyl on the absorption spectrum of a solution of lactose, orcinol and concentrated hydrochloric acid

Fig. 1(*e*). Effect of acid concentration on the extinction at 670 mμ: curve A, sucrose; curve B, lactose

Fig. 1(*f*). Calibration curve for 0 to 200 μg of lactose with the correlation line shown

Fig. 1(*g*). Deviation from linearity of the calibration curve for high concentrations of lactose. The straight-line part of the curve is the correlation line from Fig. 1(*f*)

It can be seen that the presence of ferric ions is essential for the production of the green chromogen, whereas the brown chromogen is apparently formed in the absence of ferric ions. This effect is more clearly seen in Fig. 1 (*d*), which gives the absorption spectrum obtained after heating for 15 minutes a mixture of 3 ml of the concentrated hydrochloric acid, 0·5 ml of a 10 per cent. solution of orcinol, 1 ml of a solution of lactose (100 μg per ml in water) and 0·1 ml of a 0·1 per cent. solution of 2:2′-dipyridyl. The position of the maximum in the absence of ferric ions appears to be somewhat displaced from that of the corresponding peak obtained in their presence.

Concentration of acid—The green chromogen is formed only in the presence of concentrated acid. Mixtures of 0·5 ml of a 1 per cent. solution of ferric chloride in water, x ml of concentrated hydrochloric acid, $(3 - x)$ ml of water, 0·5 ml of a 10 per cent. solution of orcinol and 1 ml of a solution of lactose (100 μg per ml) were boiled for 6 minutes in a water bath. After the solution had cooled, 2 ml of ethanol were added and the extinctions at 670 mμ were measured. Fig. 1 (*e*) shows the results obtained with the lactose solution and also with a solution of sucrose (1000 μg per ml).

Optimal conditions—The conditions adopted for the determination of lactose in protein-free solutions were heating for 10 minutes in a boiling-water bath 3 ml of a 0·1 per cent. solution of ferric chloride in concentrated hydrochloric acid, 1 ml of the sample (containing 0 to 200 μg of lactose per ml.) and 0·5 ml of a 10 per cent. solution of orcinol in absolute ethanol. After cooling, 2 ml of absolute ethanol were added and the absorption at 670 mμ was measured. A typical calibration curve is shown in Fig. 1(*f*); at higher concentrations of lactose than mentioned above, the response deviates from linearity, as shown in Fig. 1(*g*).

With the procedure described, two levels of lactose were used to determine the variability of the method; 6 samples at each level of lactose were taken. The mean values, with their standard errors, were $106 \pm 0{\cdot}6$ μg per ml and $56 \pm 1{\cdot}2$ μg per ml. The standard errors quoted are less than 3 per cent. of the corresponding mean values.

Application of the method to milk and to mammary tissue suspensions—

The determination of lactose in milk or in suspensions of mammary gland in general involves the prior precipitation of protein. Ferric hydroxide has often been used as a protein precipitating agent[8,14] and was used in the early stages of this present work.

Samples of whole cow or rat milk (obtained after prior injection of 5 i.u. of oxytocin) were treated with colloidal ferric hydroxide to precipitate the protein. Lactose was determined in aliquots of the filtrate. Two methods of determination were used; first, the orcinol method described here and, secondly, the chloramine-T method of Hinton and Macara,[15] as modified by Folley and Greenbaum.[8] In general the two methods gave closely similar results (see Table I), but occasionally, even on clear milk filtrates, higher lactose titres were obtained by the chloramine-T method than by the orcinol method. Typical results are shown in Fig. 2, which illustrates the variability between the two methods.

Table I

Comparison of the orcinol method with other methods for determining lactose

Sample	Lactose found by orcinol method with ferric hydroxide used as precipitant for protein, μg per ml	Lactose found by alternative method with ferric hydroxide used as precipitant for protein, μg per ml
Cows' milk	100·0	100·0, 108·0, 103·5, 100·0 by chloramine-T method
	100·0	101·6,[a] 99·6,[b] 99·5[c] by orcinol method
	100·0 $\pm$ 2·8*	97·4 $\pm$ 0·66 by chloramine-T method,* 99·4 $\pm$ 0·66 by Benedict's method*
Rat mammary gland	100·0	109·0, 105·0, 102·0, 96·0 by methylamine method
	100·0	152·4 $\pm$ 8·9 by chloramine-T method†

* Mean and standard error of 6 determinations.
† Mean and standard error of 26 determinations.
[a] Zinc hydroxide used as precipitant for protein.
[b] Trichloroacetic acid used as precipitant for protein.
[c] Tungstophosphoric acid used as precipitant for protein.

In a similar series of experiments, 1 in 10 homogenates of rat or rabbit mammary gland were prepared by adding 9 volumes of ice-cold glass-distilled water to 1 volume of gland and homogenising in a top-driven Waring Blendor.[16] The suspension was strained through muslin to remove intractable connective tissue. Aliquots of the suspension were deproteinised with colloidal ferric hydroxide and lactose was determined in the filtrate by both the orcinol and the chloramine-T methods. From Table I it can be seen that almost invariably the chloramine-T method gave appreciably higher results. The ratio of the results by the two methods was variable from animal to animal, indicating that some factor in the methods was not under strict control. The precipitation stage seemed the obvious cause of the discrepancies.

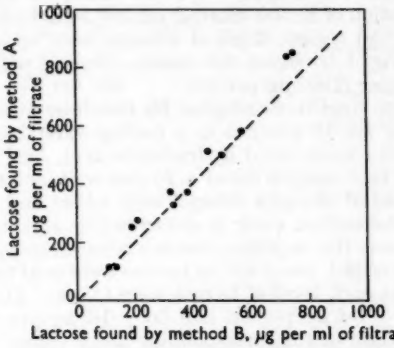

Fig. 2. Correlation between results by the chloramine-T method (method A) and the orcinol method (method B). The broken line represents the theoretical agreement between the two methods

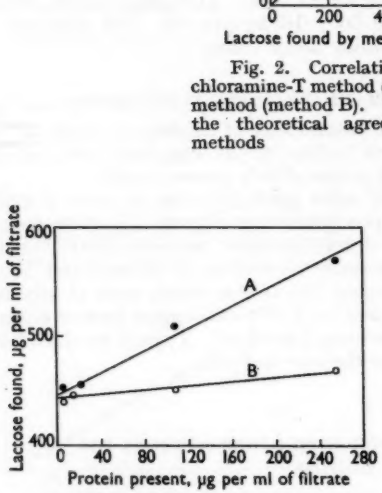

Fig. 3(*a*). Effect of protein on the determination of lactose in milk filtrates: curve A, chloramine-T method; curve B, orcinol method

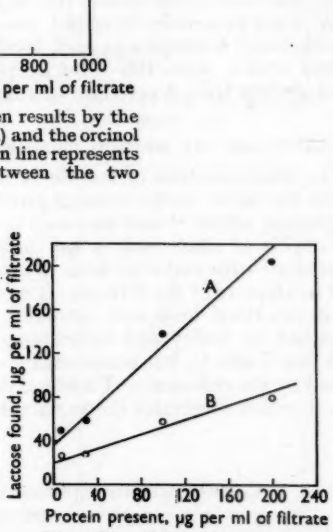

Fig. 3(*b*). Effect of protein on the determination of lactose in tissue suspensions of rat mammary gland: curve A, chloramine-T method; curve B, orcinol method

It was thought probable that the higher values obtained by the chloramine-T method were the result of incomplete precipitation of protein from the milk or mammary gland suspensions by ferric hydroxide, the soluble protein remaining in the filtrate affecting the chloramine-T method more than the orcinol method. This was considered likely from the study of the effects of protein on both methods of lactose determination. The protein in both mammary tissue suspensions and in milk was precipitated to various degrees by adding increasing amounts of colloidal ferric hydroxide. After the protein had been removed by filtration, a series of more or less opalescent solutions was obtained, in which the protein was determined by the colorimetric method of Lowry, Rosebrough, Farr and Randall.[17] Determinations of lactose by both the chloramine-T and the orcinol methods were carried

out on aliquots of these suspensions. Fig. 3 (*a*) shows that for milk the chloramine-T method was affected by the presence of protein; increasing concentrations of protein increased the "apparent lactose content." The orcinol method did not appear to be affected to any appreciable degree by small concentrations of protein. It therefore appeared that the difference between the two methods of determination was due to incomplete precipitation of protein, possibly albumin, by ferric hydroxide. When protein is completely removed from milk filtrates, or when the methods are compared on aqueous solutions of lactose, the two methods agree, as can be seen from Table I.

However, although the complete removal of protein from mammary gland suspensions does decrease the difference between the two methods of determination, even with protein-free filtrates of mammary gland the methods gave different results (see Figure 3(*b*) and Table I). The chloramine-T method gave consistently higher results than the orcinol method. It seems probable that this was the result of some reducing component of mammary suspensions interfering under the mild conditions of the chloramine-T reaction and leading to falsely high results, rather than of a component of the mammary gland inhibiting the orcinol reaction, which involves hydrolysis by boiling concentrated hydrochloric acid. The same discrepancy between the chloramine-T and orcinol methods occurred with precipitants other than ferric hydroxide, *e.g.*, zinc hydroxide, although usually the disagreement was not so great, as the filtrates were virtually protein-free. However, it can be seen from Table I that the methylamine method of Malpress and Morrison[7] gave results similar to those by the orcinol method when used on rat mammary gland suspensions.

Various other protein precipitating agents were used in lactose determinations by the orcinol method on milk or mammary gland suspensions. Both the zinc hydroxide reagent of Letonoff[18] and 5 per cent. trichloroacetic acid were found to be satisfactory. However, a disadvantage in the routine use of zinc hydroxide is that relatively large amounts are required to deproteinise milk or mammary gland suspensions completely. Trichloroacetic acid (5 per cent.) is a satisfactory precipitant for both milk and mammary gland suspensions and does not interfere with the orcinol reaction.

Determinations were carried out on aqueous solutions of lactose with and without the addition of trichloroacetic acid to a final concentration of 10 per cent. The results show that, compared with a value of 100 μg per ml of lactose obtained in the absence of trichloroacetic acid, the mean of 8 determinations of lactose in the presence of trichloroacetic acid was 99·9 $\pm$ 1·1 μg per ml.

Complete recovery of added lactose from milk or mammary tissue suspensions was obtained by using ferric hydroxide or zinc hydroxide as precipitant, and the orcinol method for the determination (see Table II).

TABLE II

RECOVERY OF ADDED LACTOSE FROM MILK AND TISSUE SUSPENSIONS OF MAMMARY GLAND

Sample	Lactose added,	Lactose recovered by chloramine-T method		Lactose recovered by orcinol method	
	μg	μg	%	μg	%
Cows' milk	30·9	29·9[a]	97·0	30·0[a]	97·0
	56·5	54·9[a]	97·2	60·0[a]	106·0
	84·7	79·2[a]	93·4	85·0[a]	100·3
	141·2	136·0[a]	96·3	135·5[a]	96·0
	38·1	38·0,[a] 38·0[b]	99·7, 99·7		
	76·0	81·0,[a] 72·0[b]	106·6, 94·7		
Rat mammary gland	61·9			65·0[a]	105·0
	113·0			112·0[a]	99·2
		Mean	98·1		100·5
		Standard error	$\pm$1·4		$\pm$1·6

[a] Ferric hydroxide used as precipitant for protein.
[b] Zinc hydroxide used as precipitant for protein.

Procedure for determining lactose—A 1-ml sample of milk was deproteinised by adding trichloroacetic acid to a final concentration of 5 per cent. and the volume was made up to

250 ml with water. The solution was filtered and lactose was determined in 1-ml portions of the clear filtrate as described under "Optimal conditions," p. 821. The volumes stated can be scaled down proportionately as required. For tissue suspensions of rat mammary gland, 2 to 5 ml of a 1 in 10 water homogenate were deproteinised by adding trichloroacetic acid to a final concentration of 5 per cent. and the volume was made up to 25 ml with water. The solution was filtered and 1-ml portions of the filtrate were taken for the determination of lactose as before.

ABSORPTION CURVES OF OTHER CARBOHYDRATES—

Various 1-ml samples of other carbohydrates were substituted for lactose in the procedure given under "Optimal conditions," p. 821. The absorption spectra of these solutions are shown in Fig. 4. It can be seen that all the carbohydrates tested, with the exception of xylose, gave similar absorption spectra. Xylose showed no peak near 540 mμ. The orcinol method therefore cannot be used to determine lactose in the presence of other carbohydrates, except when they are present in trace amounts.

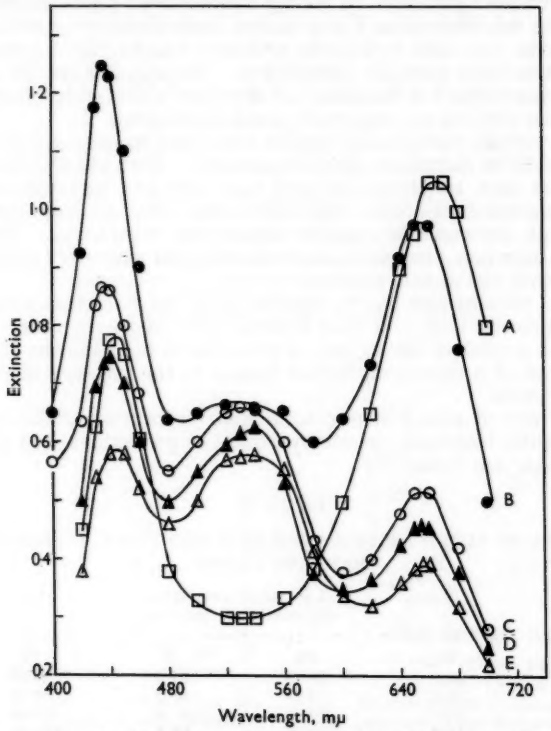

Fig. 4. Absorption spectra: curve A, 100·0 μg of xylose per ml; curve B, 203·8 μg of galactose per ml; curve C, 207·4 μg of glucose per ml; curve D, 190·6 μg of maltose per ml; curve E, 196·3 μg of sucrose per ml. Extinction values were measured with a Unicam SP500 spectrophotometer in 1-cm cells

DISCUSSION

The method described is suitable for the rapid determination of microgram amounts of lactose in milk or in mammary tissue suspensions. An advantage of the method is that it is insensitive to traces of protein, in contrast to the chloramine-T method. The orcinol method readily determines 10 μg of lactose.

Brückner[19] has described the reaction of disaccharides with orcinol in concentrated sulphuric acid, but his work is not suitable for the routine determination of lactose, as the time

of heating is short and critical. The advantage of the orcinol reaction described here is that none of the reaction variables is used in a critical region.

I express my gratitude to Dr. A. L. Greenbaum for his help and encouragement of this work and to Mr. L. McCollum and Mr. D. Planterose for skilled assistance. I also express my gratitude both to the Agricultural Research Council for a grant during the early stages of this work and to the Beit Memorial Trust for the award of a Fellowship.

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Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Method for the Determination of Synthetic Detergents

Synthetic Detergents

AFTER considering the available methods of determination, the Ministry of Housing and Local Government Committee on Synthetic Detergents only recommended a method for the anionic type, namely, that of Longwell and Maniece.[1] The A.B.C.M. - S.A.C. Joint Committee, however, considered that it would be helpful to describe methods for the non-ionic and cationic types, since these are used in industry, although they comprise only about 5 per cent. of the total domestic and industrial usage. Apart from the Longwell and Maniece method, the remaining methods have therefore been included *for information* and it must be emphasised that they should be regarded as tentative only.

The methods of Epton[2] and of Barr, Oliver and Stubbings[3] were originally devised for the determination of anionic surface-active agents present in fairly high concentration in synthetic-detergent preparations. By a reversal of the technique of Epton's method (Method A) it is possible to determine cation-active materials. Apart from results by the method of Longwell and Maniece, any estimate of surface-active compounds (in low concentration) is open to doubt when suspended solids are present.

ANIONIC DETERGENTS

(LOW CONCENTRATIONS)

PRINCIPLE OF METHOD—

In this method, due to Longwell and Maniece,[1] a complex of the detergent with methylene blue in alkaline solution is extracted with chloroform. After the chloroform extract has been washed with an acid solution of methylene blue, it is compared colorimetrically with standards prepared from Manoxol O.T.

RANGE—

For contents of anion-active material from 20 to 150 μg.

REAGENTS—

Alkaline phosphate solution—Dissolve 10 g of analytical-reagent grade anhydrous disodium hydrogen phosphate in distilled water. Adjust the pH to 10 by adding sodium hydroxide solution and dilute to 1 litre with distilled water.

Neutral methylene blue solution—Dissolve 0·35 g of methylene blue, B.P., in distilled water and dilute to 1 litre.

Acid methylene blue solution—Dissolve 0·35 g of methylene blue, B.P., in about 500 ml of distilled water, add 6·5 ml of sulphuric acid, sp.gr. 1·84, and dilute to 1 litre with distilled water.

Chloroform—Analytical-reagent grade.

Standard solution of anion-active agent—Dissolve 0·100 g of Manoxol O.T. (sodium dioctylsulphosuccinate) in distilled water and dilute to 1 litre.

Dilute 10 ml of this solution to 100 ml with distilled water. Prepare this solution freshly as required.

1 ml $\equiv$ 10 μg of Manoxol O.T.

PROCEDURE—

(*i*) Measure into a separating funnel a volume of sample preferably containing between 20 and 150 μg of anion-active material. (It is generally impracticable to

take more than 10 ml of crude or settled sewage, owing to emulsification on shaking with chloroform, but it is possible to take up to 100 ml of good-quality effluent when the detergent content is very low.) If sulphide is present, proceed as described in Note 1. Dilute the contents of the funnel to 100 ml with distilled water. Add 10 ml of alkaline phosphate solution, 5 ml of neutral methylene blue solution and 15 ml of chloroform.

(*ii*) Shake the mixture evenly and gently twice a second for 1 minute. Allow the layers to separate, breaking up any emulsion formed in the funnel by gentle agitation with the flattened end of a glass rod. Run the clear chloroform layer into a second separating funnel containing 100 ml of distilled water and 5 ml of acid methylene blue solution. Rinse the first funnel with 2 ml of chloroform added from a burette and then run this rinsing into the second funnel.

(*iii*) Shake the contents of the second funnel as before and allow the layers to separate. Run the chloroform layer through a small filter-funnel plugged with cotton-wool moistened with chloroform into a 50-ml calibrated flask, rinsing with a further 2 ml of chloroform.

(*iv*) Repeat operations (*ii*) and (*iii*), using two 10-ml portions of chloroform. Dilute the combined extracts in the flask to the mark with chloroform. (See Note 2.)

Measure the optical densities of the chloroform extract and of the blank in a spectrophotometer or an absorptiometer, using a wavelength of 6500 A in a spectrophotometer, or a suitable orange filter if an absorptiometer is used. Read the number of micrograms of detergent, in terms of anion-active material, equivalent to the observed optical densities of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of detergent in the sample.

Establish the calibration graph as follows—

Treat appropriate amounts of dilute Manoxol O.T. solution covering the range 20 to 200 μg as for the test solution and determine the optical densities. Construct a graph relating the optical density to the concentration.

NOTES—1. *Sulphide interference*—Sulphide, if present, must be oxidised before extraction. Place the required volume of sample in the first separating funnel, and add 10 ml of alkaline phosphate solution and 2 ml of 20-volume hydrogen peroxide. Allow the mixture to stand for 5 minutes and then dilute it to 110 ml with distilled water. Add 5 ml of neutral methylene blue solution and 15 ml of chloroform, and continue as from paragraph (*ii*) of the procedure.

2. Before a further determination is carried out, the separating funnels should be rinsed with dilute nitric acid to remove adsorbed methylene blue.

ANIONIC DETERGENTS

(HIGH CONCENTRATIONS)

Two methods are described.

Method A[2] has some advantage with slightly opalescent samples. Reversal of the technique of this method may be used for estimating cation-active materials (see p. 829).

Method B[3] is applicable to clear solutions and has a direct end-point.

METHOD A

PRINCIPLE OF METHOD—

This method utilises the fact that cation-active materials decompose the chloroform-soluble complex formed between anion-active materials and methylene blue, water-soluble methylene blue being liberated.

RANGE—

For contents of anion-active agents above 10 mg per litre of sample.

REAGENTS—

Chloroform B.P.

Methylene blue solution—Dissolve 0·003 g of methylene blue, B.P., and 5 g of anhydrous sodium sulphate in 100 ml of distilled water containing 1·2 per cent. of sulphuric acid, sp.gr. 1·84.

Standard solution of anion-active agent (*Manoxol O.T.*)—Dissolve 2·22 g of Manoxol O.T. in 1 litre of distilled water.

Solution of cation-active agent (*cetylpyridinium bromide*)—Dissolve 1·54 g of cetylpyridinium bromide in 1 litre of distilled water.

PROCEDURE—

Standardisation of cetylpyridinium bromide solution—Transfer by pipette 10·0 ml of the standard solution of Manoxol O.T. to a 250-ml glass-stoppered flask. Add 25 ml of the methylene blue solution and 15 ml of chloroform. Shake the flask with just sufficient force to ensure that the phases mix thoroughly. At this stage the upper layer is pale blue and the lower layer is dark blue. From a burette add the cetylpyridinium bromide solution 2 ml at a time with intermittent shaking. When the colour of the upper layer begins to deepen, reduce the rate of addition. The end-point is reached when both layers are the same colour when viewed in reflected light.

Titration of sample—In a similar manner, titrate a suitable aliquot of the effluent sample with the standardised cetylpyridinium bromide solution. The volume of sample taken should preferably contain approximately the equivalent of 0·02 g of Manoxol O.T. (or approximately 0·005 *M* with respect to its anion-active agent content).

Express the anion-active agent content of the sample as milligrams per litre in terms of Manoxol O.T.

METHOD B

PRINCIPLE OF METHOD—

In this method the anion-active material in the sample is titrated with standard cation-active material. The end-point is marked by the appearance of a blue colour due to the formation of a complex between the excess cation-active material and bromophenol blue, this complex being soluble in chloroform.

RANGE—

For contents of anion-active agent above 10 mg per litre of sample.

REAGENTS—

Chloroform, B.P.

Bromophenol blue indicator solution—A 0·04 per cent. solution in 20 per cent. aqueous ethanol.

Standard solution of anion-active agent (*Manoxol O.T.*)—Dissolve 0·444 g of Manoxol O.T. in 1 litre of distilled water.

Solution of cation-active agent (*cetyltrimethylammonium bromide*)—Dissolve 0·364 g of cetyltrimethylammonium bromide in 1 litre of distilled water.

PROCEDURE—

Standardisation of cetyltrimethylammonium bromide solution—Transfer by pipette 25·0 ml of the standard solution of Manoxol O.T. to a glass-stoppered flask and add 100 ml of distilled water, 50 ml of chloroform and 5 drops of bromophenol blue indicator solution. Titrate the mixture with cetyltrimethylammonium bromide solution, shaking after each addition. In the early stages of the titration, the chloroform emulsifies in the aqueous phase, but ready separation into two layers occurs as the titration proceeds, particularly as the end-point is approached. Allow about 1 minute to elapse between successive additions, in 0·1-ml increments towards the end-point, which is taken as the point at which the first indication of blue colour appears in the chloroform layer. The blue colour intensifies with further additions of cetyltrimethylammonium bromide solution.

Titration of sample—In a similar manner, titrate a suitable aliquot of the effluent sample with the standardised cetyltrimethylammonium bromide solution. The volume of sample taken should contain approximately the equivalent of 0·01 g of Manoxol O.T.

Express the anion-active agent content of the sample as milligrams per litre in terms of Manoxol O.T.

CATIONIC DETERGENTS

(CONCENTRATIONS GREATER THAN 10 mg PER LITRE)

As mentioned in the introductory paragraphs, a reversal of the technique described under Anionic Detergents, Method A, may be used to estimate cation-active materials.

REAGENTS—

As for Anionic Detergents, Method A, but with the cetylpyridinium bromide as the standard.

PROCEDURE—

Standardisation of Manoxol O.T. solution—Transfer by pipette to a 250-ml glass-stoppered flask 10·0 ml of standard cetylpyridinium bromide solution. Add 25 ml of methylene blue solution and 15 ml of chloroform. Shake the flask with just sufficient force to ensure thorough mixing of the two phases. At this stage the upper layer is dark blue and the lower layer is pale blue.

From a burette add the solution of Manoxol O.T. 1 ml at a time with intermittent shaking, allowing 1 minute to elapse between successive additions. When the colour of the lower layer begins to deepen, reduce the rate of addition. The end-point is reached when both layers are the same colour when viewed in reflected light.

Titration of sample—In a similar manner, titrate a suitable aliquot of the effluent sample with the standard solution of Manoxol O.T. The volume of sample taken should preferably contain approximately the equivalent of 0·015 g of cetylpyridinium bromide. From the standard titration and the sample calculate the number of milligrams of cation-active material present and express the result in terms of milligrams of cetylpyridinium bromide per litre of sample.

CATIONIC DETERGENTS

(CONCENTRATIONS LESS THAN 10 mg PER LITRE)

A method of direct estimation cannot, as yet, be recommended. However, a method depending upon the reduction of anionic activity has given reasonable results.[4] It is offered here tentatively.

PRINCIPLE OF METHOD—

When anion-active and cation-active materials are mixed, in solution, mutual neutralisation of the activities takes place. However, the decrease in apparent anionic activity is not exactly proportional to the amount of cationic detergent present, but falls off as the amount of cationic material is increased in the presence of a constant amount of anionic material.

PROCEDURE—

First obtain an approximation (in mg per litre) of the concentration of cation-active material in the effluent sample, as follows—

Shake various aliquots of the sample with an aqueous solution of anion-active material of known concentration (about 15 mg per litre). Treat these mixtures, and the pure solution of the anion-active material, according to Degens's single acid-extraction method for anion-active material[5]: in this method (see below), the free anion-active material forms with methylene blue a coloured complex, which is extracted by chloroform. The approximate concentration of cation-active material in the sample is then assessed by selecting, by means of simple visual inspection of the chloroform extracts, that aliquot of sample showing a reduction by half or more of the colour produced by the solution of anion-active material alone.

Proceed to estimate more closely the concentration of cation-active material present. Into a series of separating funnels measure, respectively, 10-ml portions of solutions containing amounts of standard cation-active material around the

approximate value of the sample in steps of −2, −1, +1 and +2 mg per litre. Into another separating funnel measure the previously ascertained volume of the effluent sample. Shake the contents of each funnel with 10 ml of a solution of standard anion-active material having a known concentration of about 15 mg per litre. Proceed to estimate, in each case, the uncombined anion-active material by Degens's method. The reduction in anionic activity effected by the sample can then be related to that effected by the standard cation-active material, and so assessed.

NOTE—Certain ions, *e.g.*, nitrate and chloride, interfere with Degens's method. If these are present, they should be present in the standard in the same concentration as in the sample.

Degens's method—In a small separating funnel put 10 ml of chloroform, 100 ml of distilled water, 10 ml of the anion-active test solution and 5 ml of acid methylene blue solution (see under "Reagents," Anionic Detergents, (*a*), Longwell and Maniece method). Shake the mixture for 1 minute, at the rate of 2 shakes per second. Extract three times with 10-ml portions of chloroform, filtering each chloroform extract through the same plug of cotton-wool into a 50-ml calibrated flask, and dilute the solution to the mark with chloroform. Measure the optical density by any suitable instrument that has been calibrated in terms of standard anion-active material, *e.g.*, Manoxol O.T., treated as above.

NON-IONIC DETERGENTS

(TENTATIVE METHOD)

The method[6,7] described below may be found useful for the determination in trade effluents of non-ionic polyoxyethylene-type detergents (*e.g.*, Lissapol N) when the concentration is greater than 10 mg per litre of sample; at concentrations below this the method is not reliable, and even at the higher concentrations it should be accepted with some reserve, particularly when the protein content is high.

PRINCIPLE OF METHOD—

The non-ionic detergent is determined by precipitation of a detergent - barium molybdophosphate complex from an aqueous ethanolic solution of the ether extract of the sample. The precipitate is either (*i*) dissolved in ethylene glycol monomethyl ether (methyl Cellosolve) and hydrochloric acid, and its optical density determined in an ultra-violet spectrophotometer at a wavelength of 3100 A, or (*ii*) digested with a mixture of sulphuric and perchloric acids, and the phosphate determined colorimetrically.

NOTE—This method can be applied direct to good-quality effluents, but, for those with a high protein content, a preliminary deproteinisation with zinc sulphate and barium hydroxide is usually necessary. However, deproteinisation leads to appreciable loss of non-ionic detergent, presumably owing to adsorption by the precipitate. This can be allowed for by applying a correction factor of 1·4, but results obtained on such deproteinised samples must be regarded as approximate.

Owing to the very small amounts of solids precipitated, their separation from liquids is effected by spinning in a centrifuge and all operations subsequent to the extraction with ether and before the colour measurement are carried out in one and the same centrifuge tube.

RANGE—

For non-ionic detergent (active agent) contents above 10 mg per litre of sample.

REAGENTS—

Barium chloride solution—Dissolve 10 g of barium chloride, $BaCl_2.2H_2O$, in 90 ml of distilled water.

Molybdophosphoric acid solution, 1 per cent. w/v.

Zinc sulphate solution—Dissolve 5 g of zinc sulphate, $ZnSO_4.7H_2O$, in distilled water and dilute to 100 ml.

Barium hydroxide solution—To 4·75 g of barium hydroxide, $Ba(OH)_2.8H_2O$, add 100 ml of distilled water and boil gently, with stirring, for 2 minutes. Filter hot through filter-paper into a bottle; keep the bottle well stoppered to minimise the formation of barium carbonate.

Hydrochloric acid, diluted (1 + 1)—Mix equal volumes of hydrochloric acid, sp.gr. 1·18, and distilled water.

Hydrochloric acid, dilute (1 + 9).

Acid ethylene glycol monomethyl ether—To 400 ml of the freshly distilled reagent add 100 ml of diluted hydrochloric acid (1 + 1), mix well and cool.

Acid ammonium molybdate solution—Dissolve 10 g of ammonium molybdate, $(NH_4)_5Mo_7O_{24}.4H_2O$, in 100 ml of distilled water, pour the solution into a well cooled mixture of 150 ml of sulphuric acid, sp.gr. 1·84, and 150 ml of distilled water, and mix well. Protect the solution from light.

Metol - sulphite solution (*Tschopp's reagent*)—Dissolve 40 g of sodium metabisulphite and 1 g of sodium sulphite, $Na_2SO_3.7H_2O$, in cold distilled water, add 0·2 g of Metol (*p*-methylaminophenol sulphate) and stir well until dissolved. Dilute the solution to 100 ml and mix well.

Perchloric acid, 60 per cent. w/v.

Sulphuric acid, diluted (1 + 1)—Mix carefully equal volumes of sulphuric acid, sp.gr. 1·84, and distilled water and cool the solution.

Sodium chloride.

Diethyl ether.

Aqueous ethanol, 20 per cent. v/v.

Sodium hydroxide solution, N.

*Lissapol N active agent**—For preparation of standard solutions.

PROCEDURE—

Clear effluents—

Weigh 15 g of sodium chloride into a dry 100-ml separating funnel with a short stem. Pour into the funnel 40·0 ml of the sample. Add 10 ml of distilled water and 10 ml of ether measured with a graduated cylinder, place the stopper in the funnel and shake vigorously until the sodium chloride has almost completely dissolved. Allow the mixture to stand for at least 5 minutes. Run the aqueous layer into a 100-ml beaker. Swirl the ether layer gently in the funnel and allow to stand for another minute. Run off the extra 0·5 ml or so of aqueous layer into the beaker containing the rest of the aqueous layer. Into the neck of the funnel insert a rubber bung carrying two narrow glass tubes, the first of which reaches just inside the rubber bung and is bent at an angle of about 60° to the vertical outside the funnel, and the second of which reaches right to the bottom of the funnel and is bent at an angle of 120° to the vertical outside the funnel. This second tube is drawn out to a fairly coarse jet at each end. By blowing into the first tube, expel about 3 to 4 ml of the ether extract into a 15-ml graduated centrifuge tube, which contains a small piece of porous pot and which is immersed for about half its length in water in a 250-ml beaker. Warm the beaker on the top of a steam-bath until the ether boils *gently*. When all the ether has evaporated, blow in another 3 to 4 ml and repeat the evaporation in stages until all the ether has been removed from the funnel. Return the separated aqueous layer from the 100-ml beaker to the funnel and extract again with 10 ml of ether, separating and evaporating the ether extract as before.

Wash down the sides of the tube with 3 ml of aqueous ethanol. Warm the tube in boiling water until the contents begin to boil. Remove from the water bath and add successively 1 ml of diluted hydrochloric acid (1 + 1), 0·5 ml of barium chloride solution and 2 ml of molybdophosphoric acid solution. Stir the contents of the tube with a glass rod until homogeneous and wash down the rod with 1 or 2 ml of dilute hydrochloric acid (1 + 9), allowing the washings to run into the tube. Remove the tube from the bath and allow it to stand for at least 2 hours, preferably overnight.

Dilute to the 10-ml mark with dilute hydrochloric acid (1 + 9) and spin in a centrifuge for 5 minutes at about 1500 r.p.m. and 20-cm radius. Using a "wash-bottle" fitting

* Subsequently referred to as Lissapol NX.

similar to that used in transferring ether from the funnel to the tube, remove the supernatant liquid. For this operation, lower the end of the long tube until it is level with the 0·4-ml mark in the centrifuge tube. Collect the expelled liquid in another centrifuge tube and examine it carefully for suspended matter. If any is present, indicating disturbance of the precipitate, return the liquor to the precipitation tube, again spin in a centrifuge and remove in a similar manner the liquor into the second tube. Pour about 2 ml of dilute hydrochloric acid (1 + 9) into the tube and disperse the precipitate thoroughly by stirring with a thin glass rod. Wash down the rod into the tube with 1 or 2 ml of dilute hydrochloric acid (1 + 9) and then fill the tube to the 10-ml mark with dilute hydrochloric acid (1 + 9). Spin in a centrifuge and remove the supernatant liquid as before.

Determine the amount of Lissapol NX in the precipitate indirectly by measuring its molybdophosphoric acid content in either of the following ways.

Ultra-violet spectrophotometric method—Pour into the centrifuge tube containing the precipitate about 4 to 5 ml of acid ethylene glycol monomethyl ether. Disperse the precipitate with a thin glass rod and stir the contents of the tube until solution is complete or nearly so. Wash down the rod into the tube with 1 or 2 ml of the reagent and fill the tube to about the 9-ml mark. Pour its contents into a 25-ml calibrated flask and wash out with further 10-ml and 5-ml portions of the reagent, adding the washings to the flask. Dilute to the mark with acid ethylene glycol monomethyl ether and mix well.

Measure the optical density of the solution in a 1-cm cell at 3100 A, using a spectrophotometer with the acid ethylene glycol monomethyl ether reagent as reference liquid. From a calibration graph (see later) read the concentration of active agent (in mg per litre), in terms of Lissapol NX, corresponding to the observed optical density.

Colorimetric molybdenum blue method (*see Note* 1)—To the centrifuge tube containing the washed precipitate add 1·0 ml of diluted sulphuric acid (1 + 1) in such a way as to wash down the sides of the tube; then add 0·2 ml of perchloric acid and 0·5 ml of *N* sodium hydroxide solution. Warm the contents of the tube for a few seconds on a steam-bath and pour the mixture into a 25-ml beaker. Wash the tube with several small amounts of hot distilled water, adding the washings to the beaker. Evaporate the contents of the beaker until fumes appear. Allow the contents to cool, add 7 ml of distilled water and heat on a steam-bath for 10 to 15 minutes. Filter the solution through a 9-cm Whatman No. 42 filter-paper into a 8-inch × 1¼-inch boiling-tube. Rinse the beaker with several small amounts of hot distilled water, pouring each successive amount of water over the filter-paper to wash it thoroughly; collect the filtered washings in the boiling-tube. Continue this procedure until the volume of solution in the tube is 25 ml. Add 5·0 ml of acid ammonium molybdate and 5·0 ml of Metol - sulphite solution. Immerse the tube in a boiling-water bath for 30 minutes and then cool the contents to room temperature.

Transfer the contents of the tube to a 50-ml calibrated flask and dilute to the mark with distilled water. Measure the optical density of the solution in a 4-cm cell, using a spectrophotometer at a wavelength of 6500 A, or in an absorptiometer with a suitable red filter. Use water in the comparison cell. From a previously prepared calibration graph (see later) read the concentration, in mg per litre, of Lissapol NX corresponding to the observed optical density.

Alternatively, the colours may be compared with proprietary coloured discs in a suitable instrument; for example, the B.D.H. Nessleriser, and Tschopps's phosphate disc C, can be used after the following procedure: transfer the cooled solution to a Nessler cylinder and dilute to 50 ml with distilled water; fill the comparison tube with a solution prepared by heating together 5 ml of acid ammonium molybdate solution, 5 ml of Metol - sulphite solution, 25 ml of distilled water and 1 ml of diluted sulphuric acid (1 + 1), for 30 minutes, as in an actual test.

The following concentrations of Lissapol NX have been found to correspond to the disc markings—

Amount of phosphate (read from disc), μg	15	30	45
Lissapol NX (40-ml sample without deproteinisation), mg per litre	5	10	15
Lissapol NX (40-ml sample with deproteinisation), mg per litre ..	7	14	21

Poor-quality effluents and crude sewages—

Measure from a 100-ml graduated cylinder 80 ml of the sample into a 250-ml beaker. Add from a graduated cylinder 10 ml of 5 per cent. zinc sulphate solution, swirl to mix the solution, and then add slowly, with swirling, 10 ml of barium hydroxide solution. Heat just to boiling, cool rapidly to room temperature, and then filter through a 12·5-cm filter-paper (Whatman No. 1 paper is suitable). Measure 50·0 ml of the clear filtrate (see Note 2) into a separating funnel containing 15 g of sodium chloride and proceed as described under "Clear effluents" from "Add 10 ml of ether measured with a graduated cylinder, . . ."

PREPARATION OF CALIBRATION GRAPHS—

Standard solution of Lissapol NX—Into a 100-ml beaker weigh 0·40 ± 0·01 g of Lissapol NX. Add about 20 ml of distilled water and stir, with gentle heating, until the material is freely dispersed. Allow to cool and then dilute with water to 1 litre in a calibrated flask. The weight of Lissapol NX present in 1 ml of this solution if present in a 40-ml sample (see Note 2) of effluent would correspond to a concentration of 10 mg of Lissapol NX per litre.

(*a*) *Preparation of graph for use with samples that have not been deproteinised before extraction with ether*—Into a series of 15-ml centrifuge tubes measure x ml of standard Lissapol NX solution (x = 0·5, 1·0, 1·5, 2·0, 2·5 and 3·0) from a burette, $(3 - x)$ ml of distilled water, 0·5 ml of absolute ethanol, 1 ml of diluted hydrochloric acid (1 + 1), 0·5 ml of barium chloride solution and 2·0 ml of molybdophosphoric acid solution. Then proceed as described under "Clear effluents," from "Stir the contents of the tube with a glass rod . . ." Construct a graph relating the Lissapol NX content—expressed as mg per litre on a 40-ml test sample—to the optical density, measured either with an ultra-violet spectrophotometer or with an absorptiometer, as may be appropriate.

(*b*) *Preparation of graph for use with samples that have been deproteinised with zinc sulphate and barium hydroxide before extraction with ether*—Measure carefully into a series of 250-ml beakers from a burette x ml of standard Lissapol NX solution (x = 1, 2, 3, 4 and 6) and $(80 - x)$ ml of water. Then proceed as described under "Poor quality effluents and crude sewages," from the words "Add 10 ml of 5 per cent. zinc sulphate solution . . ." Construct a graph relating the Lissapol NX content—expressed as mg per litre on a 40-ml test sample—to the optical density measured either with an ultra-violet spectrophotometer or with an absorptiometer, as may be appropriate.

NOTES—1. This method for Lissapol N-type detergents depends on the determination of the phosphate content of the Lissapol N - barium - molybdophosphoric acid complex. The weight of complex isolated from unit weight of Lissapol NX is constant. It has therefore been assumed that the ratio of phosphorus to Lissapol NX in the complex is also constant.

2. Fifty millilitres of the deproteinised filtrate are equivalent to 40 ml of the original sample.

3. Most domestic detergents contain diluents in the form of inorganic salts or water or both, the active agent content not being disclosed. In consequence, the normal practice in recent publications on this subject is to express the detergent content as mg of active agent per litre and *not* mg of the detergent as marketed per litre. This practice has been followed in the foregoing method.

ANION- AND CATION-ACTIVE SUBSTANCES IN ADMIXTURE

(TENTATIVE METHOD)

When anionic and cationic surface active agents are present together, the foregoing methods will give only a measure of the extent to which one is in excess of the other.

The following methods[8] are intended to measure the total content of each form: however, apart from the use of a resin to separate the two forms, no new principle or method is involved.

NOTE—The column of resin may give a blank value of up to 1 p.p.m. of anion-active material on being washed with 100 ml of water; the washings may be tinted violet.

(*a*) Determination of anion-active material in presence of cation-active material

The following method is appropriate no matter which form is present in excess.

Principle of method—

When a solution containing anion- and cation-active substances is passed through a column of a cation-exchange resin, for example, Zeo-Karb 225, the cation-active material is absorbed.

Procedure—

In a tube 1 cm in diameter prepare a column of resin 30 cm long; this will serve for several estimations.

Pass 20 ml of the sample through the column, taking 20 minutes for the operation. Wash the column with three 20-ml portions of distilled water; the time of passage for the last washing can be reduced to 10 minutes.

(*b*) Estimation of cation-active material in presence of an excess of anion-active material

Procedure—

(*i*) A determination of anion-active material is carried out directly on the sample, by one of the methods previously described.

The difference between this result and that obtained in (*a*) above gives a very approximate indication of cationic content equivalent to the difference in anionic strength by the two methods.

(*ii*) A more correct estimate is obtained by passing a portion of sample through a resin column as in (*a*). Then treat the percolate with a standard solution of cation-active material until the same result is obtained as in the direct estimation on the sample. A fairly good estimate in terms of cation-active material can thus be obtained.

(*c*) Estimation of cation-active material in presence of a slight excess or less anion-active material

Again remove the cationic material by passing the sample through a resin column as before and determine the anion-active material alone. This amount of anion-active material is then included in the excess used to determine the cation-active material directly on the sample by the method given for determination of Cationic Detergents.

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Notes

POLAR AND STERIC EFFECTS IN PAPER CHROMATOGRAPHY

THE general dependence of chromatographic behaviour on chemical structure is probably inaccessible, but certain irrefragable examples of simple relations exist and occasionally these suggest aspects of the chromatographic process that have a significant effect on its over-all kinetics. In adsorption chromatography, the effect of molecular size, steric hindrance of polar groups, inter- and intra-molecular hydrogen bonding, inductive effects, planarity of molecules, the presence of unsaturations and rings, and other structural features have been studied.[1 to 11] Partition between phases is the dominant physical process in paper chromatography.[12,13,14] Consequently an additivity in the effect of certain groupings is to be expected.[12,15] Function $R_M \equiv \log_{10}[(1 - R_F)/R_F]$ gives straight line plots with the number of methylene groups in aliphatic mono- and dicarboxylic acids[16,17,18] and mono- and diamines,[19] and similar regularities have been observed for hydroxy compounds,[15] flavones,[20] carbohydrates[15,21,22] and amino acids.[23,24] The study of additivity regularities has been developed to a high pitch in the work of Schauer and Bulirsch[25,26] and in the dipole moment - R_F investigations of Franc and Jokl.[27] Chelation,[20] group position,[28] ionic radii[29] and optical isomerism[30,31] are examples of other atomic or molecular features that have been considered,[14] but not much work relating general molecular characteristics to chromatographic movement on paper has appeared.

The discovery of structural correlations, from the great mass of published data on chromatography, is complicated by large variations in R_F values, not only with solvent systems,[32,33,34] but also with temperature,[32,35] paper and the direction of its fibres,[32,36] and even with geometrical features of the system.[37,38,39,40,41] Difficulties associated with the widely differing conditions under which results are obtained have to some extent been surmounted in the comprehensive survey of benzene derivatives effected by Franc and Latinák.[42,43,44] These authors indicate that linear relations exist between dipole moments of benzene and naphthalene derivatives and their R_F values.[42,44]

AN EXAMPLE OF THE INFLUENCE OF POLAR EFFECTS

Dipole moments give the over-all polarity of molecules, but the electron-withdrawing effect of a substituent, in the *meta* or *para* position, to an original polar group in a substituted benzene,

TABLE I

σ AND R_F VALUES OF SUBSTITUTED ANILINES WITH STATIONARY POLAR PHASES

Substituent	σ	R_F values in different solvents—* 1	2	3	4	5
p-NH_2†	−0·660	0·25	0·75	0·26	0·70	0·46
p-OH†	−0·357	0·51	0·82	0·33	0·74	0·46
m-NH_2†	−0·161	0·62	0·75	0·35	0·71	0·50
m-Me†	−0·069	0·89	0·85	0·87	0·86	0·83
H†	0·0	0·88	—	0·71	0·91	0·84
m-MeO‡	0·115	0·82	0·82	—	—	0·80
p-Cl‡	0·227	0·88	0·88	—	—	0·86
m-F‡	0·337	—	0·90	—	—	—
m-Cl‡	0·373	0·90	0·89	—	—	0·89
m-NO_2‡	0·710	0·85	0·86	—	—	0·85
p-NO_2‡	0·778	0·84	0·85	—	—	0·81
p-MeO‡	−0·268	0·81	0·81	—	—	0·79
p-CO_2H†	0·265	0·88	0·56	0·79	0·41	0·71
m-CO_2H†	0·355	0·81	0·42	0·78	0·45	0·76

* The solvents have the following compositions—

Solvent	Methanol, %	*iso*Amyl alchol, %	Benzene, %	Water %
1	40	20	20	20
2	35	17·5	35	12·5
3	35	17·5	35	12·5 (2 *N* HCl)
4	35	17·5	35	12·5 (4% of NH_3)
5	30·8	15·2	46	8

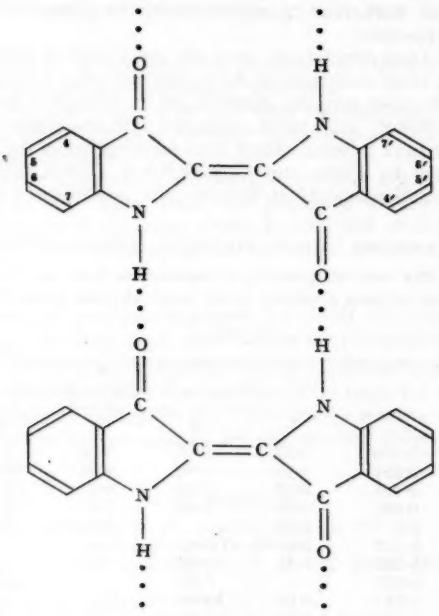
† Data given by Ekman.[45]

‡ Results obtained under similar conditions to those used by Ekman except that the spots were located by spraying first with ice-cold nitrous acid (prepared by adding 1 ml of a 0·5 per cent. sodium nitrite solution to 4 ml of 0·1 *N* hydrochloric acid at 0° C), followed by lightly spraying with a 1 per cent. solution of 2-naphthol in 0·25 *N* sodium hydroxide.

is related to the Hammett σ value[46] of the substituent. If the polarity of the original group largely determines partition between the phases, then some regularity should exist between the σ value of the substituent and the R_F value in a series of compounds. This has been found to occur in substituted anilines. When the polar phase is stationary, R_F values at first rise and subsequently fall with increase in σ value, as shown in Table I. Reciprocal behaviour in reversed-phase systems is shown by the following data, which are given by Micheel and Schweppe[47]—

Substituent	*p*-NH_2	*p*-Me	*m*-NH_2	*m*-Me	*m*-No_2	*p*-NO_2
σ	−0·660	−0·170	−0·161	−0·069	0·710	0·778
R_F	0·28	0·22	0·16	0·17	0·21	0·28

It is of interest to note that expected variations from the regular pattern occur with the *p*-methoxy group and with the amino acids. The *p*-methoxy group frequently exhibits anomolous behaviour in σ correlations[48] and the many types of interaction between the amino and carboxyl groups[49] would lead to departures from the main scheme. Relationships between σ and R_F values are not, however, general features of benzenoid compounds and tend to be completely obscured in poly-substitution.



(I)

TABLE II

R_F VALUES OF INDIGOID DYES

Compound	Group length*	R_F†	R_F‡
Indigo	1·38	0·07	0·0
5:5′:6:6′-Tetrachloroindigo ..	1·38	0·0	0·0
7:7′-Dichloroindigo	3·49	0·88	with front
7:7′-Dibromoindigo	3·83	0·92	with front
4:4′-Dichloroindigo	3·49	0·92	0·0
4:4′-Dibromoindigo	3·83	0·92	with front
4:4′-Di-iodoindigo	4·15	0·95	with front

* Distance from the centre of the adjacent-ring carbon atom to the external point of the group, measured in the direction of the C–R axis.[50]

† Solvent: pyridine - *n*-butanol - water (60:20:20).

‡ Solvent: pyridine - *n*-butanol - water (10:10:40).

An example of the influence of steric effects

If a particular group, by virtue of its polarity or hydrogen bonding propensities for example, plays a major role in determining partition between two phases, then steric inhibition of this group should profoundly affect the R_F value of the compound. This hypothesis is supported by the pronounced variation in the chromatographic behaviour of indigoid dyes as shown in Table II. Indigo (**I**) and indigos substituted in the 5 and 6 positions are inter-molecularly bonded,[51] possess very low solubility in both polar and non-polar solvents, and migrate very slowly in chromatographic systems. Large substituents in positions 4 or 7 sterically prevent this association and must minimise interaction with the polar phase. These compounds, therefore, partition mainly into the non-polar phase and possess high R_F values. These results have interesting relevance to the use of indigoid dyes in mapping intracellular-esterase distributions.[51]

We are indebted to the Department of Scientific and Industrial Research for a special research grant.

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July 25th, 1957

Book Reviews

ELEMENTARY QUANTITATIVE ANALYSIS: THEORY AND PRACTICE. By W. J. BLAEDEL and V. W. MELOCHE. Pp. xvi + 826. Evanston, Illinois: Row, Peterson & Company. 1957. Price $6.90.

Any new textbook on quantitative inorganic analysis must pass the acid test of being superior in content or presentation of subject matter to the many excellent books now in use. With this criterion in mind it must be adjudged that the present work is impressive, and deserves close examination by all those whose concern is the teaching of quantitative inorganic analysis at university and technical college level.

Briefly, the text is divided into four sections with a supplement for advanced students. The chapters are presented on a numerically classified unit system, and the link-up of the supplement with the relevant parts of the foregoing sections is thus easily found. The introductory section, comprising the first five chapters, deals with the general organisation of quantitative analysis. As in many other American books of this kind, a treatment of the mathematics essential to the science is given. There is also an excellent discussion of the theory of errors in its simpler aspects, including methods for the control, assessment and reduction of errors from various sources. Section II, which is divided into 8 chapters covering 167 pages, deals with gravimetric analysis. Attention is first of all drawn to the fundamental principles of gravimetry, with considerable stress laid on the mode of operation of the classical free-swinging balance. The factors that affect the formation of precipitates are well brought out and discussed. Considerable stress is laid on methods for solving numerical problems. Section III, which has 7 chapters and covers 265 pages, is concerned with titrimetric analysis. Again the fundamental principles and apparatus are first of all reviewed, and, as in the previous section, considerable attention is paid to the calibration and method of using apparatus. In place of the conventional opening with acid - base work, precipitation titrations are first considered, presumably to link up with the earlier gravimetry. Acid - base equilibria and methods follow, while the remaining two chapters treat of redox phenomena. Section IV, dealing with special topics, consists of three chapters covering 53 pages. In the first of these, attention is devoted to the principles governing the absorption of light by chemical systems. Subsequently, the apparatus and technique associated with colorimetry, absorptiometry and spectrophotometry are dealt with. The second chapter discusses a few aspects of instrumentation in no great detail, and the third chapter purports to be a guide to the literature of analytical chemistry. Here one feels that the authors have been somewhat narrow-minded in their outlook. Only American journals and abstracts are cited and of the 64 standard reference books listed for the further guidance of the student only 7 have been published outside the U.S.A.

The supplement is an advanced discussion of many points already mentioned in less detail in earlier sections. It includes problems of a numerical character and review questions, but no experiments. This section is modern in conception and adds tremendously to the value of the book.

Theory and practice are interwoven throughout the text, with the experiments appearing at the end of most chapters. There are only 29 experiments described in this book of 826 pages. Personally the reviewer feels that the book is top heavy with theory, but this feeling is tempered by the excellent presentation of the theory and the manner in which working techniques are described. However, in analysis, more than in any other branch of chemistry, it is essential that the student should have practical knowledge at the bench, and not only book learning.

With this reservation in mind, I have no hesitation in recommending the book very strongly. It is delightfully readable, which is an outstanding and unusual property in any textbook.

T. S. WEST

SYNTHETIC ION-EXCHANGERS: RECENT DEVELOPMENTS IN THEORY AND APPLICATION. By G. H. OSBORN, F.R.I.C. Pp. x + 194. London: Chapman & Hall Ltd. 1955. Price 30s.

During the last decade, growth of interest in ion-exchange has been both rapid and widespread; new materials have become generally available and new techniques have been developed. The appearance of the volume under review is, therefore, very welcome, particularly since some hundred or so of its pages are devoted to a classified bibliography covering the five years before publication.

The remainder of the book (some ninety pages) is devoted to a brief account of the development of ion-exchange methods during the period covered by the bibliography, together with an account of the properties of commercially available synthetic materials. Some portions of this are more successful than others. The sections devoted to analytical aspects of the subject, to ion-exchange

membranes and to the technique of ion-exclusion are excellent and readable accounts, although in the first of these there appears to be some confusion between electric current and potential in the description of Manecke's work on ionophoresis. The early chapters of the book are, however, less satisfactory. The sections on Exchange Equilibria and Kinetics, for example, are inadequate for any real understanding of the theoretical principles involved, and yet the inclusion of mathematical relationships such as those given on p. 12 is not likely to be of much value to the average reader without a much fuller account of their derivation. Although generally well produced, the book contains a number of minor errors suggesting that proof-reading might have been more thorough.

JOHN ALLEN

QUANTITATIVE METHODS OF ORGANIC MICROANALYSIS. By S. J. CLARK, Ph.D., A.R.I.C. Pp. x + 253. London: Butterworths Scientific Publications. 1956. Price 30s.

This book describes the methods of quantitative organic microanalysis employed in the laboratories of British Nylon Spinners Ltd., in Birmingham University and in some other industrial and research organisations. Rather more than one half of the volume is occupied by the description of methods of ultimate analysis and the determinations of functional groups and physical constants. The remaining chapters are devoted for the most part to discussion of the microchemical balance, general laboratory technique, titration in non-aqueous media and the determination of phosphorus, arsenic and various metals.

Brief reference to the most interesting features of this volume is all that can be attempted within the limits of a short review.

For the determination of carbon and hydrogen the "Rapid" or "Empty tube" combustion method and a modified version of Friedrich's method are described. In addition to the Dumas method, the more recent Kirsten combustion procedure for the determination of nitrogen is described, and its several advantages are indicated. A recent modification of the Kjeldahl method that eliminates the distillation stage is described. In this procedure ammonia in the digest is determined volumetrically after reaction with excess of sodium hypochlorite.

The determination of sulphur and halogens is described in an interesting chapter incorporating much of the recent work carried out at Birmingham University. Methods described for the determination of these elements in the absence of fluorine include the "Rapid" combustion, the Schöberl and the sodium peroxide bomb-fusion methods. The application of 4-amino-4'-chlorodiphenyl hydrochloride, and of EDTA to the completion of the determination of sulphur in some cases, is described. A separate section of this chapter is devoted to the description of methods for the determination of fluorine and the other halogens, either individually or in any combination. In all methods the fluorine-containing substance is decomposed by fusion with potassium or sodium in a specially designed bomb, and the fluorine is determined by titration with thorium nitrate or by precipitation as lead chlorofluoride, according to the amount present. The inclusion of an ion-exchange method in the chapter on the determination of metals, and of a chapter devoted to titration in non-aqueous media, will be welcomed by many readers.

Although the usefulness of the book would be enhanced by an extension of the chapter on the determination of functional groups, and some additions to the methods for determination of molecular weight, it cannot be criticised on these grounds, in view of the author's statement of the purpose of the book given in the preface.

The methods described are for the most part as up to date as can reasonably be expected in a continuously developing branch of chemical analysis, the subject matter is clearly and concisely presented and the diagrams are adequate. Apart from the omission of one letter on p. 142, and some ambiguity in the statement of the magnitude of the blank value on p. 151, no misprints or errors were detected.

Dr. Clark has made a welcome addition to the relatively few books of this type, and there is little doubt that it will provide much of interest and value to the practising microanalyst.

A. F. COLSON

INORGANIC MICROANALYSIS: QUALITATIVE AND QUANTITATIVE. By R. BELCHER, Ph.D., D.Sc.; F.R.I.C., F.Inst.F., and C. L. WILSON, Ph.D., D.Sc., F.R.I.C., F.I.C.I. Second Edition. Pp. x + 153. London, New York and Toronto: Longmans, Green & Co. Ltd. 1957. Price 21s.

This book is presented as the second edition of the same authors' "Qualitative Inorganic Microanalysis," which was published in 1946 at the price of 2s. 6d. with the intention that junior

students could have a book on the subject within their financial reach (reviewed in *Analyst*, 1946, 71, 452). The new edition has greater pretensions and is a logically self-contained whole, covering both qualitative and quantitative aspects of inorganic microanalysis. The first edition is represented by the first 69 pages of this book; the subject matter has, however, been extended and revised. The scheme for cation identification without the use of hydrogen sulphide has been reproduced in the form given in the first edition and has also been modified to allow the detection of cerium, molybdenum, thorium, titanium, tungsten, uranium, vanadium and zirconium. These ions have not been included in the more usual scheme in which hydrogen sulphide is used. The identification of anions has also been dealt with in an original and helpful manner.

The second part of the book, which describes customary quantitative methods of analysis, begins with a chapter on the balance and its proper use for microanalysis. Here instruction is given for using not only the microbalance, but also the ordinary analytical balance when used for centigram work. There are four chapters on appropriate apparatus, which may often be made by the student himself. Representative determinations have been selected to train the student in gravimetric, titrimetric and electrochemical methods, with use of the apparatus previously described. One appendix lists the reagents required for the qualitative analyses described in the text, and another those for the quantitative analyses; a third appendix give s a brief guide to the literature on inorganic microanalysis. The student trained from this book should acquire a good basic knowledge of the subject and its practical skills. It is suggested, however, that it would have been well to provide him also with the minimum of theory to support the practical text: for instance, equations are necessary on p. 127 to show why one iodate ion liberates six equivalents of iodine (a precendent, if needed, is provided on p. 129, where the equation is given for the interaction of the triple acetate of sodium with sodium hydroxide). G. H. WYATT

Publications Received

COSMETICS: SCIENCE AND TECHNOLOGY. Edited by EDWARD SAGARIN, H. D. GOULDEN, EMIL G. KLARMANN and DONALD H. POWERS. Pp. xx + 1433. New York and London: Interscience Publishers Inc. 1957. Price $25.00; 188s.

A SHORT COURSE IN QUANTITATIVE ANALYSIS. By HOBART H. WILLARD, N. HOWELL FURMAN and EGBERT K. BACON. Second Edition. Pp. vi + 243. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Co. Inc. 1957. Price $4.75; 35s.

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MICRODIFFUSION ANALYSIS AND VOLUMETRIC ERROR. By EDWARD J. CONWAY, M.D., D.Sc., F.R.I.C., F.R.C.P.I., F.R.S. Fourth Edition. Pp. xviii + 465. London: Crosby Lockwood & Son Ltd. 1957. Price 42s.

ION-EXCHANGE RESINS. By J. A. KITCHENER. Pp. viii + 109. London: Methuen & Co. Ltd.; New York: John Wiley & Sons Inc. 1957. Price 9s. 6d.

METHODS OF BIOCHEMICAL ANALYSIS. Volume V. Edited by DAVID GLICK. Pp. x + 502. New York and London: Interscience Publishers Inc. 1957. Price $9.50; 75s.

FREE RADICALS IN SOLUTION. By CHEVES WALLING. Pp. xii + 631. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1957. Price $14.50; 116s.

BACTERIAL FERMENTATIONS. By H. A. BARKER. Pp. viii + 95. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1957. Price $3.00; 24s.

The first book in the series: CIBA Lectures in Microbial Biochemistry.

TECHNOLOGY FOR SUGAR REFINERY WORKERS. By OLIVER LYLE. Third Edition. Pp. 663. London: Chapman & Hall Ltd. 1957. Price 70s.

SOLVENT EXTRACTION IN ANALYTICAL CHEMISTRY. By GEORGE H. MORRISON, Ph.D., and HENRY FREISER, Ph.D. Pp. xii + 269. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1957. Price $6.75; 54s.

Erratum

SEPTEMBER (1957) ISSUE, p. 639, Table I, formula of the last compound.
For "$(C_3H_7{\cdot}CH_2{\cdot}O)_3PO$" *read* "$(C_3F_7{\cdot}CH_2{\cdot}O)_3PO$."

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